National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

GENDER DIMORPHISM IN KETOPROFEN PHARMACOKINETICS IN RATS

BY

EUGENIA LYNN PALYLYK

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY.**

IN

PHARMACEUTICAL SCIENCES (PHARMACOKINETICS)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

FALL 1994

National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-95245-8

Canada

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR:	EUGENIA LYNN PALYLYK
TITLE OF THESIS:	GENDER DIMORPHISM IN KETOPROFEN PHARMACOKINETICS IN RATS
DEGREE:	DOCTOR OF PHILOSOPHY
YEAR THIS DEGREE GRANTED:	FALL 1994

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

P.O. Box 278
Smoky Lake, Alberta, Canada T0A 3C0

Date: AUGUST 25, 1994

The Goal

Each life converges to some centre
Expressed or still;
Exists in every human nature
A goal,

Admitted scarcely to itself, it may be,
Too fair
For credibility's temerity
To dare.

Adored with caution, as a brittle heaven,
To reach
Were hopeless as the rainbow's raiment
To touch,

Yet persevered toward, surer for the distance;
How high
Unto the saints' slow diligence
The sky!

Ungained, it may be, by a life's low venture,
But then,
Eternity enables the endeavoring
Again.

Emily Dickinson (1830-1886)

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **GENDER DIMORPHISM IN KETOPROFEN PHARMACOKINETICS IN RATS** submitted by **EUGENIA LYNN PALYLYK** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in Pharmaceutical Sciences (Pharmacokinetics).

Dr. F. Jamali (Supervisor)

Dr. Y.K. Tam

Dr. F.M. Pasutto

Dr. R.T. Coutts

Dr. A.S. Russell

Dr. M.E. Morris (External Examiner)

Date: AUGUST 25, 1994

This thesis is dedicated to my parents,
who have through their unending support and guidance
instilled in their children the desire and commitment to attain
goals and respect that knowledge belongs to a vast
family of whom many are dead, few are living
and countless numbers yet unborn

ABSTRACT

Chiral nonsteroidal antiinflammatory drugs (NSAID)s such as ketoprofen play an essential role in the therapy of inflammation and chronic arthropathies. A gender dimorphism in the stereoselective pharmacokinetics of ketoprofen was studied in Sprague Dawley rats following *i.v.* administration of racemic ketoprofen. The plasma AUC of S-ketoprofen was almost double and the amount of S-ketoprofen glucuronide recovered in urine was almost four times greater in female (39.6 ± 18.2 %) than male rats (8.2 ± 5.8%).

The enantiomers of ketoprofen were highly bound (>95%) to both male and female rat plasma and stereoselectivity or dimorphism in binding was not evident. In both sexes, renal excretion of S-ketoprofen glucuronide and liver or kidney microsomal glucuronidation of ketoprofen enantiomers were altered by gonadectomy with or without concurrent estradiol or testosterone hormonal replacement. Glucuronidation by male rat liver microsomes was substantially higher than from female microsomes whereas glucuronidation by female rat kidney microsomes was higher than those from male rats. There was no stereoselectivity in glucuronidation. Significant correlations were found between the amount of S-ketoprofen glucuronide formed by liver ($r = -0.859$) or kidney ($r = 0.597$) microsomes and the cumulative 24 h urinary recovery of S-ketoprofen glucuronide in these rats.

Despite the pharmacokinetic dimorphism, there was no gender

difference in the increased urinary excretion of ^{51}Cr-EDTA, a marker of small intestinal permeability changes, following an oral dose of ketoprofen in rats.

The interaction of probenecid and ketoprofen was studied following development of a stereospecific reverse-phase HPLC assay. A probenecid concentration dependency and gender difference in the extent of interaction of probenecid with S-ketoprofen in the rat were found. Biliary recovery of *i.v.* administered R- or S-ketoprofen glucuronides was almost 50% lower in the presence than absence of probenecid. Addition of probenecid to microsomal incubations resulted in a significant decrease in liver but not kidney glucuronidation of ketoprofen in both sexes.

The data suggest that the disposition of NSAIDs such as ketoprofen may be under hormonal control, thus, the large interindividual variability reported in studies of these agents may be reduced or explained by distinguishing between sex in experimental protocols.

ACKNOWLEDGEMENTS

Firstly, I wish to express my sincere gratitude to Dr. Fakhreddin Jamali for the supervision extended to me over the course of the research project. His unending enthusiasm towards science and research will always be remembered.

I would also like to thank the members of my Graduate Committee - Dr. Yun K. Tam, Dr. Franco M. Pasutto, Dr. Ronald T. Coutts and Dr. Anthony S. Russell for their guidance and assistance at various stages of my graduate program.

My very special thanks and appreciation are extended towards the graduate students of the Faculty of Pharmacy; in particular, my many colleagues in our laboratory for the friendship, help and invaluable sharing of ideas and enlightening conversations throughout the years.

I would also like to acknowledge the support and very special friendships of Sheree Madro, Janet Dietrich, Cathy Lemko, Brian Phillips, Dr. Silvia Alessi-Severini and Dr. Derrick and Glenda Freitag.

My most special and heartfelt thanks are extended to my parents, Orest and Grace Palylyk, for the unending support, advice, love and patience I have been blessed to be the recipient of throughout the years. To my sister, Dr. Claudia Palylyk, thank you for the constant support and encouragement and most specially for having gone first and giving unselfishly of your experience.

And to Mitch, for your love that has brought so much to my life - anything I could write would fall short of how much you mean to me. You have filled me with the anticipation of enduring happiness and a future filled with every good and wonderful thing imaginable.

In addition, I would sincerely like to thank the Medical Research Council of Canada, the Alberta Heritage Foundation for Medical Research and Merck-Frosst, Canada for providing me with graduate studentships throughout the course of my studies.

TABLE OF CONTENTS

Page

1. INTRODUCTION 1

1.1. Gender differences 1

1.1.1. Absorption 2

1.1.2. Distribution 3

1.1.3. Metabolism 4

1.1.4. Excretion 7

1.2. Ketoprofen 8

1.2.1. Chemistry of ketoprofen 10

1.2.2. Analytical methods 11

1.2.3. Basic pharmacology 14

1.2.3.1. Antiinflammatory action 15

1.2.3.2. Analgesic action 15

1.2.3.3. Antipyretic action 16

1.2.3.4. Other properties 16

1.2.3.5. Enantiospecific activity 17

1.2.4. Pharmacokinetic studies in humans 17

1.2.4.1. Absorption 18

1.2.4.2. Distribution 19

1.2.4.3. Metabolism 20

1.2.4.4. Excretion 22

1.2.5. Pharmacokinetic studies in animals 23

1.2.5.1. Rats 23

1.2.5.2. Other animal species 24

1.2.6. Side effects and toxicity 24

1.2.7. Drug interactions 27

1.3. Rationale for study of gender differences in the stereoselective pharmacokinetics of ketoprofen in rats 28

1.4. Hypotheses 30

1.5. Specific objectives and their rationale 31

1.5.1. Study the gender dimorphism in pharmacokinetics of ketoprofen enantiomers in rats 31

1.5.1.1. Determine gender and tissue differences in glucuronidation of ketoprofen enantiomers . 31

1.5.1.2. Determine effect of gonadectomy and sex hormones 32

1.5.1.3. Determine gender differences in plasma protein binding 32

1.5.1.4. Determine gender differences in increased permeability of ^{51}Cr-EDTA as a marker of intestinal toxicity 33

1.5.2. Study effect of probenecid on the stereoselective pharmacokinetics of ketoprofen in male and female rats 33

1.5.2.1. Establish a suitable stereospecific method for simultaneous quantification of ketoprofen enantiomers and probenecid in biological samples 34

1.5.2.2. Determine the stereoselectivity and concentration dependence of the interaction of probenecid and ketoprofen enantiomers 34

1.5.2.3. Determine effect of probenecid on the disposition of ketoprofen enantiomer glucuronide conjugates 34

1.5.2.4. Determine effect of probenecid on glucuronidation of ketoprofen enantiomers . 35

2. EXPERIMENTAL 36

2.1. Chemicals 36

2.2. Assays 37

2.2.1. Apparatus and chromatographic conditions 37

2.2.2. Standard solutions 37

2.2.3. Sample preparation and analysis 37

2.2.3.1. Ketoprofen assay 37

2.2.3.2. Ketoprofen and probenecid assay 38

2.2.3.2.1. Extraction yields 39

2.2.3.2.2. Derivatization yields 40

2.2.3.2.3. Standard curves and accuracy .. 41

2.2.3.2.4. Other compounds 41

2.3. *In vivo* studies in rats 42

2.3.1. Surgical methods 42

2.3.1.1. Jugular vein cannulation 42

2.3.1.2. Bile-duct cannulation 42

2.3.1.3. Ovarectomy 43

2.3.1.4. Castration 43

2.3.2. Drug administration and sample collection 44

2.3.2.1. Pharmacokinetics in female and male rats ... 44

2.3.2.1.1. Intact rats 44

2.3.2.1.2. Bile-duct cannulated rats 45

2.3.2.2. Gonadectomy and sex hormones 45

2.3.2.2.1. Ketoprofen 45

2.3.2.2.2. Sex hormones 47

2.3.2.3. Intestinal permeability study 47

2.3.2.4. Probenecid and ketoprofen pharmacokinetics 48

2.3.2.4.1. Female rats 48

2.3.2.4.2. Male rats 49

2.3.2.5. Probenecid and ketoprofen glucuronide pharmacokinetics 49

2.3.2.5.1. Intact rats 49

2.3.2.5.2. Bile-duct cannulated rats 50

2.4. *In vitro* studies 51

2.4.1. Protein binding 51

2.4.1.1. Apparatus and techniques 51

2.4.2. Glucuronidation 52

2.4.2.1. Microsomal preparation 52

2.4.2.2. Microsomal incubation 53

2.4.2.2.1. Ketoprofen 53

2.4.2.2.2. Ketoprofen and probenecid 54

2.5. Data analysis 55

2.5.1. Pharmacokinetic indices 55

2.5.2. Statistical analysis 56

3. RESULTS 59

3.1. Assays 59

3.1.1. Ketoprofen assay 59

3.1.2. Ketoprofen and probenecid assay 59

3.1.2.1. Extraction yields 62

3.1.2.2. Derivatization yields 64

3.1.2.3. Standard curves and accuracy 64

3.1.2.4. Other compounds tested 64

3.2. Gender dimorphism in pharmacokinetics of ketoprofen in rats 66

3.2.1. Pharmacokinetic studies in female and male rats 66

3.2.1.1. Intact rats 66

3.2.1.2. Bile-duct cannulated rats 70

3.2.2. *In vitro* glucuronidation of ketoprofen 76

3.2.2.1. Liver microsomal activity 76

3.2.2.2. Kidney microsomal activity 79

3.2.3. Gonadectomy and sex hormones 83

3.2.3.1. Female rats 83

3.2.3.2. Male rats 83

3.2.4. Plasma protein binding 88

3.2.5. Intestinal permeability 91

3.3. Interaction of probenecid and ketoprofen in rats 91

3.3.1. Pharmacokinetics in female rats 91

3.3.2. Pharmacokinetics in male rats 103

3.3.3. Role in *in vivo* disposition of ketoprofen glucuronide conjugates 106

3.3.3.1. Intact rats 106

3.3.3.2. Bile-duct cannulated rats 107

3.3.4. Role in *in vitro* glucuronidation of ketoprofen 111

3.3.4.1. Liver microsomal acitivity 111

3.3.4.2. Kidney microsomal activity 112

4. DISCUSSION 115

4.1. Gender dimorphism in the stereoselective pharmacokinetics of ketoprofen in rats 115

4.1.1. Disposition in rats 115

4.1.2. *In vitro* glucuronidation by rat microsomes 120

4.1.3. Gonadectomy and sex hormones 124

4.1.4. Plasma protein binding 127

4.1.5. Intestinal permeability 128

4.2. Concentration dependent interaction of probenecid and ketoprofen enantiomers in rats 130

4.2.1. Stereospecific assay 130

4.2.2. Disposition in rats 131

4.2.3. *In vivo* disposition of ketoprofen glucuronides 137

4.2.4. *In vitro* glucuronidation by rat microsomes 140

5. CONCLUSIONS 142

6. REFERENCES 149

LIST OF TABLES

Table I Stereospecific high performance liquid chromatographic assays for ketoprofen .....Page 13

Table II Pharmacokinetic indices of ketoprofen enantiomers after various routes of administration to male rats. Data are presented as the mean ± (SD) .....Page 25

Table III Sample size and mean ± (SD) weight of rats used in the *in vivo* pharmacokinetic studies.....Page 46

Table IV Typical regression equations for R-ketoprofen, S-ketoprofen and probenecid (1-40 μg/mL) together and alone in plasma and urine samples. .....Page 63

Table V Evaluation of accuracy for ketoprofen and probenecid assay .....Page 65

Table VI Gender differences in mean ± (SD) pharmacokinetic indices of ketoprofen enantiomers in the rat. The letter (a) represents a significant difference (female *vs* male); (b) represents a significant difference (intact *vs* bile-duct cannulated) $\alpha = 0.05$ .....Page 68

Table VII Duncan's Multiple Range Test results for data in **Figures 10** and **12**.....Page 78

Table VIII Duncan's Multiple Range Test Results for differences between groups for data in **Figures 14** and **15** ..... Page 87

Table IX Plasma and urine pharmacokinetic indices after administration of 10 mg/kg *i.v.* rac-ketoprofen and various doses of probenecid in female rats. Data are presented as the mean ± (SD). Statistical analyses are in **Table X** ..... Page 94

Table X Duncan's Multiple Range Test results for data in **Table IX** .....Page 95

Table XI Correlation of R-ketoprofen and S-ketoprofen plasma concentrations with probenecid plasma concentrations at individual sampling times in female rats ..... Page 98

Table XII S:R-ketoprofen ratios in plasma and urine for each probenecid dose and MRT in female rats. Data are presented as the mean ± (SD) .....Page 99

Table XIII Duncan's Multiple Range Test results for data in **Table XII** .....Page 100

Table XIV Plasma and urine pharmacokinetic indices after administration of 10 mg/kg *i.v.* rac-ketoprofen and 0 (saline) or 100 mg/kg probenecid in male rats. Data are presented as the mean ± (SD) (n = 4 rats) .....Page 105

Table XV Summary of S-ketoprofen pharmacokinetic parameter differences between female and male rats. The symbol (♀) represents female rats and (♂) represents male rats ... Page 143

LIST OF FIGURES

Figure 1 Structure of ketoprofen ..... Page 11

Figure 2 The major metabolite of ketoprofen in humans and rats ..... Page 21

Figure 3 HPLC profiles of blank plasma (A); plasma spiked with 10 μg/mL of each enantiomer of ketoprofen and probenecid (B) and a 6h plasma sample after administration of an *i.v.* dose of 50 mg/kg probenecid and 10 mg/kg rac-ketoprofen in a female rat (C). Peaks: 1, R-internal standard; 2, S-internal standard; 3, R-ketoprofen; 4, S-ketoprofen; 5, probenecid ..... Page 60

Figure 4 HPLC profiles of blank urine (A); urine spiked with 5 μg/mL of each enantiomer of ketoprofen and probenecid (B), a 6-9h unhydrolyzed urine sample (C) and a 6-9h hydrolyzed urine sample (D) after administration of an *i.v.* dose of 50 mg/kg probenecid and10 mg/kg rac-ketoprofen in a female rat. Peaks: 1, R-internal standard; 2, S-internal standard; 3, R-ketoprofen; 4, S-ketoprofen; 5, probenecid ..... Page 61

Figure 5 Plasma concentration *vs* time profiles of R-ketoprofen (○) and S-ketoprofen (●) after administration of 10 mg/kg rac-ketoprofen *i.v.* in a representative intact female (A) and male (B) rat ..... Page 67

Figure 6 Cumulative urinary excretion of R-ketoprofen glucuronide (■) and S-ketoprofen glucuronide (⧅) in intact (n = 9) (A) and bile-duct cannulated (n = 3) (B) female and male rats. Data are presented as the mean ± SD. The letter (a) represents a significant difference (female *vs* male) $\alpha = 0.05$..... Page 69

Figure 7 Plasma concentration *vs* time profiles of R-ketoprofen (○) and S-ketoprofen (●) after administration of 10 mg/kg rac-ketoprofen *i.v.* in a representative bile-duct cannulated female (A) and male (B) rat. ..... Page 71

Figure 8 Cumulative biliary excretion of R-ketoprofen glucuronide (■) and S-ketoprofen glucuronide (▨) in bile-duct cannulated female and male rats. Data are presented as the mean ± SD (n = 3 rats each group). No significant differences were found (female *vs* male) $\alpha = 0.05$ ..... Page 73

Figure 9 Excretion rate into bile of R-ketoprofen glucuronide (A) and S-ketoprofen glucuronide (B) in bile-duct cannulated female (■) and male (□) rats. Data are presented as the mean ± SD (n = 3 rats) ..... Page 75

Figure 10 Glucuronidation of R-ketoprofen (■) and S-ketoprofen (□) by liver microsomes derived from control (CF), ovarectomized (OV), ovarectomized + testosterone (OVT) and ovarectomized + estradiol (OVE) treated female rats and control (CM), castrated (CS), castrated + estradiol (CSE) and castrated + testosterone (CST) treated male rats. Data are presented as the mean ± SD (n = 3 incubations) Statistics given in **Table VII**..... Page 77

Figure 11 Correlation of mean percent of S-ketoprofen dose as S-ketoprofen glucuronide recovered in 24h urine collection (n = 4 rats) and mean amount of S-ketoprofen glucuronide formed by liver microsomes (n = 3 incubations) from gonadectomized and sex hormone treated female and male rats ..... Page 80

Figure 12 Glucuronidation of R-ketoprofen (■) and S-ketoprofen (□) by kidney microsomes derived from control (CF), ovarectomized (OV), ovarectomized + testosterone (OVT) and ovarectomized + estradiol (OVE) treated female rats and control (CM), castrated (CS), castrated + estradiol (CSE) and castrated + testosterone (CST) treated male rats. Data are presented as the mean ± SD (n = 3 incubations) Statistics given in **Table VII**..... Page 81

Figure 13 Correlation of mean percent of S-ketoprofen dose as S-ketoprofen glucuronide recovered in 24h urine collection (n = 4 rats) and mean amount of S-ketoprofen glucuronide formed by kidney microsomes (n = 3 incubations) from gonadectomized and sex hormone treated female and male rats .....Page 82

Figure 14 Percent of S-ketoprofen dose as S-ketoprofen glucuronide recovered in 24 h urine collection with time from gonadectomy in control (□), ovarectomized (▨) and ovarectomized + testosterone (■) treated female rats. Data are presented as the mean ± SD. The letter (a) represents a significant difference from week 1 α = 0.05 Additional statistics given in **Table VIII** .....Page 85

Figure 15 Percent of S-ketoprofen dose as S-ketoprofen glucuronide recovered in 24 h urine collection with time from gonadectomy in control (□), castrated (▨) and castrated + estradiol (■) treated male rats. Data are presented as the mean ± SD. The letter (a) represents a significant difference from week 1 α = 0.05 Additional statistics given in **Table VIII** .....Page 86

Figure 16 Percent weight gain from week 1 following gonadectomy of (A) control (●), castrated (□) and castrated + estradiol (■) treated male rats and (B) control (○), ovarectomized (▲) and ovarectomized + testosterone (▲) treated female rats. Data are presented as the mean ± SD (n = 4 rats). The letter (i) represents an insignificant difference from week 1 α = 0.05.....Page 89

Figure 17 Percent unbound fraction of of R-ketoprofen (A) and S-ketoprofen (B) as a function of total (bound + unbound) plasma concentration in male (○) and female (●) rat plasma. Data are presented as the mean ± SD (n = 3). The letter (a) represents a significant difference from lower concentrations α = 0.05 .....Page 90

Figure 18 Percent ^{51}Cr-EDTA dose excretion into urine in 8 h in female and male rats. Data are presented as the mean ± SD for baseline (■) and following oral administration of 3 mg/kg rac-ketoprofen (▫). The letter (a) represents a significant difference between baseline and ketoprofen treated values (n = 6 rats each) α = 0.05 ..... Page 92

Figure 19 Plasma concentration *vs* time profiles for S-ketoprofen (○), S-ketoprofen with probenecid (●), R-ketoprofen (□), R-ketoprofen with probenecid (■) and probenecid (▲) following *i.v.* administration of 10 mg/kg rac-ketoprofen and 0 (saline) or 100 mg/kg probenecid in female rats. Data are presented as the mean ± SD (n = 3 rats) ..... Page 93

Figure 20 $AUC_{(0.5\text{-}24.5h)}$ *vs* dose relationship of probenecid after administration of 0-200 mg/kg probenecid and a constant dose of 10 mg/kg rac-ketoprofen *i.v.* in female rats ..... Page 96

Figure 21 Correlation of R-ketoprofen (A) or S-ketoprofen (B) and probenecid plasma concentrations at 2.0 h (A) or 1.5 h (B) sampling times following administration of 0-200 mg/kg probenecid and 10 mg/kg rac-ketoprofen *i.v.* doses in female rats ..... Page 101

Figure 22 Correlation of $AUC_{(0\text{-}24h)}$ of R-ketoprofen (A) or S-ketoprofen (B) and $AUC_{(0.5\text{-}24.5h)}$ of probenecid after administration of 0-200 mg/kg probenecid and 10 mg/kg rac-ketoprofen *i.v.* doses in female rats ..... Page 102

Figure 23 Plasma concentration *vs* time profiles for S-ketoprofen (○), S-ketoprofen with probenecid (●), R-ketoprofen (□), R-ketoprofen with probenecid (■) and probenecid (▲) following *i.v.* administration of 10 mg/kg rac-ketoprofen and 0 (saline) or 100 mg/kg probenecid in male rats. Data are presented as the mean ± SD (n = 4 rats) ..... Page 104

Figure 24 Plasma concentration *vs* time profiles for glucuronidated R-ketoprofen (○) and S-ketoprofen (▲) without probenecid and glucuronidated R-ketoprofen (●) and S-ketoprofen (▲) with probenecid after administration of ≈ 1 mg/kg R- and S-ketoprofen glucuronides and 0 (saline) or 100 mg/kg probenecid *i.v.* in female rats. Data are presented as the mean ± SD (n = 4 rats). Insert is a magnification of the first 3 h ..... Page 108

Figure 25 Plasma concentration *vs* time profiles for non-glucuronidated R-ketoprofen (○) and S-ketoprofen (▲) without probenecid and non-glucuronidated R-ketoprofen (●) and S-ketoprofen (▲) with probenecid after administration of ≈ 1 mg/kg R- and S-ketoprofen glucuronides and 0 (saline) or 100 mg/kg probenecid *i.v.* in female rats. Data are presented as the mean ± SD (n = 4 rats). Insert is a magnification of the first 3 h ..... Page 109

Figure 26 Percent glucuronidated R-ketoprofen (R-KT) (□) or S-ketoprofen (S-KT) (■) with or without probenecid (PB) recovered in 0-3 h collection of bile in female rats after administration of ≈ 1 mg/kg R- and S-ketoprofen glucuronides and 0 (saline) or 100 mg/kg probenecid *i.v.* Data are presented as the mean ± SD (n = 3 rats). The letter (a) represents a significant difference from without probenecid $\alpha = 0.05$ .....Page 110

Figure 27 Glucuronidation of R-ketoprofen (■) and S-ketoprofen (□) by liver microsomes in the presence of various concentrations of probenecid. Data are presented as the mean ± SD (n = 3 incubations). The letters (a) represent a significant difference from 0 μM probenecid concentration and (b) represents a significant difference from 0, 40 and 200 μM probenecid concentrations ..... Page 113

Figure 28 Glucuronidation of R-ketoprofen (■) and S-ketoprofen (□) by kidney microsomes in the presence of various concentrations of probenecid. Data are presented as the mean ± SD (n = 3 incubations). There were no significant differences at any probenecid concentration ..... Page 114

Figure 29 Disposition of R-ketoprofen and S-ketoprofen following *i.v.* administration of rac-ketoprofen in the rat. The following symbols represent: S_{plasma}, R_{plasma} = enantiomer in plasma; S_{conj}, R_{conj} = enantiomer glucuronide conjugate; S_{bconj}, R_{bconj} = enantiomer glucuronide conjugate eliminated via biliary excretion; $S_{intestine}$, $R_{intestine}$ = deconjugated enantiomer in intestine .....Page 144

LIST OF ABBREVIATIONS AND SYMBOLS

AUC	Area under the plasma concentration-time curve
$AUC_{(0-t)}$	Area under the plasma concentration-time curve from 0 hour to the last measured concentration
$AUC_{(0-\infty)}$	Area under the plasma concentration-time curve from 0 hour to infinity
AUC_{BC}	Area under the plasma concentration-time curve in bile-duct cannulated rats from 0 hour to 9 hours
AUC_{intact}	Area under the plasma concentration-time curve in intact rats from 0 hour to 9 hours
AUMC	Area under the first moment curve
CF	Control female rat
C_{last}	Last concentration measured
CM	Control male rat
CL_{bconj}	Biliary clearance of glucuronide conjugate
CL_{uconj}	Urinary clearance of glucuronide conjugate
CL_r	Renal clearance
CL_{TB}	Total body clearance
cm	Centimeter(s)
C_{max}	Maximum plasma concentration
CS	Castration

CSE	Castration + estradiol
CST	Castration + testosterone
CV	Coefficient of variation
ģ	Centrifugal force
g	Gram(s)
h	Hour(s)
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
H_2SO_4	Sulfuric acid
i.d.	Internal diameter
i.p.	Intraperitoneal
i.v.	Intravenous
k_a	Absorption rate constant
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
kg	Kilogram(s)
L	Liter(s)
M	Molar
mg	Milligram(s)
$MgCl_2$	Magnesium chloride
min	Minute(s)
mL	Milliliter(s)

mm	Millimeter(s)
mM	Millimolar
MQC	Minimum quantifiable concentration
MRT	Mean residence time
NaH_2PO_4	Sodium phosphate, monobasic
Na_2HPO_4	Sodium phosphate, dibasic
nm	Nanometer(s)
NaOH	Sodium hydroxide
NSAID	Nonsteroidal antiinflammatory drug
o.d.	Outer diameter
OV	Ovarectomy
OVE	Ovarectomy + estradiol
OVT	Ovarectomy + testosterone
PEG	Polyethylene glycol
pk_a	Dissociation constant
r	Correlation coefficient
r^2	Correlation coefficient
R	R enantiomer
rac	Racemic
R_{bconj}	R glucuronide conjugate eliminated via biliary excretion
R_{conj}	R glucuronide conjugate
$R_{intestine}$	R deconjugated enantiomer in intestine

R_{plasma}	R enantiomer in plasma
S	S enantiomer
s	Second(s)
S_{bconj}	S glucuronide conjugate eliminated via biliary excretion
S_{conj}	S glucuronide conjugate
SD	Standard deviation
$S_{intestine}$	S deconjugated enantiomer in intestine
S_{plasma}	S enantiomer in plasma
$t_{1/2}$	Elimination half-life
t_{max}	Time to peak concentration
UDPGA	Uridine 5′ diphosphate glucuronic acid
UDPGT	Uridine 5′ diphosphate glucuronyltransferase
ΣXu_{bile}	Cumulative recovery from bile
ΣXu_{urine}	Cumulative recovery from urine
V_d	Volume of distribution
2APA	2-arylpropionic acid
^{51}Cr-EDTA	^{51}Cr labelled ethylenediamine tetracetic acid
α	Level of significance
β	Terminal elimination rate constant
μg	Microgram(s)
μL	Microliter(s)
μM	Micromolar

1. INTRODUCTION

1.1. Gender differences

It has long been recognized that gender, phase of menstrual cycle, oral contraceptive use, menopause or andropause may pose as potential sources of variability in experimental studies (Bonate, 1991; Wilson, 1984; Giudicelli and Tillement, 1977). These factors may lead to a gender dimorphism in drug disposition and subsequently affect pharmacologic response and toxicity (Bonate, 1991; Giudicelli and Tillement, 1977).

Although reports of sex differences in pharmacokinetic parameters of drugs have been made in the past, the actual mechanism(s) underlying this dimorphism in pharmacokinetics remains poorly understood. The major reason for this is that pharmacokinetic parameters often represent a hybrid of underlying physiologic processes, many of which occur simultaneously or sequentially. As a result, elucidation of the factor or pathway under hormonal control becomes extremely difficult as is the ability to predict its effect on individual pharmacokinetic parameters.

Relatively few investigations have been specifically devoted to the influence of sex on drug pharmacokinetics in humans. It is well known that great species variations in gender related effects on drug pharmacokinetics exist (Witkamp *et al.*, 1991). The vast majority of work in this area has been done in the rat, the animal species generally displaying the most pronounced gender differences in xenobiotic metabolism and thus gender

effects on drug pharmacokinetics (Witkamp *et al.*, 1991; Chhabra and Fouts, 1974).

The following will be a brief summary of the current literature regarding the role of gender in drug pharmacokinetics in humans and rats with emphasis on the nonsteroidal antiinflammatory drugs (NSAID)s. The focus of this review is on sex differences in absorption, distribution, metabolism and excretion of drugs and not the role of gender in altered pharmacokinetics due to a pathophysiologic state or a pharmacodynamic difference such as receptor sensitivity to drugs (Kepler *et al.*, 1991; Hoffman and Levy, 1989).

1.1.1. Absorption

Many factors influence drug absorption which is often considered to be a passive phenomenon. As a result, drug absorption is dependent upon the concentration gradient of an uncharged drug moiety across the absorbing surface. Gastric acid secretion is lower in females than males (Grossman *et al.*, 1963) which in turn may affect ionization and solubility of a drug. For compounds that are actively absorbed in the gut, gender may influence the amount of carrier proteins or affinity towards substrates.

Gastric emptying time has been found to be significantly slower in women for both solids and liquids than in men (Carrio *et al.*, 1988; Datz *et al.*, 1987) and even further slowed in pregnancy and labour (Davison *et al.*,

1970). Although the exact mechanism is unknown, the authors suggest a role of the female sex hormones progesterone and estradiol in smooth muscle relaxation.

Gender differences in the disposition of the NSAIDs in humans have not been well addressed to date. The possible exception may be for salicylic acid where k_a, AUC and C_{max} were found to be lower in healthy males than females following administration of acetylsalicylic acid (ASA) (Trnavska and Trnavsky, 1983). This was attributed to enhanced formation of salicylic acid and salicyluric glucuronide conjugates during passage of the drug across the gastrointestinal membrane in males. In contrast, absorption following intramuscular injection of the lysine salt of ASA into the gluteal muscle is reported to be slower in females than males and is attributed to a higher percentage of gluteal fat in women (Aarons *et al.*, 1989).

1.1.2. Distribution

There is not much known regarding sex related differences in plasma protein binding which in turn may have an effect on drug availability, distribution and elimination. In a study by Hashimoto *et al.*, 1991 comparing amounts of 40 different serum proteins in men, premenopausal women, postmenopausal women and postmenopausal women on estrogen therapy, it was found that albumin, the major binding protein of acidic drugs like the NSAIDs, was not influenced by estrogen levels. Interestingly, a higher

association constant for binding to albumin in the female is proposed to partially explain the marked sex dependent disposition of the NSAID piroxicam in the rat (Roskos and Boudinot, 1990). In contrast to albumin, Hashimoto *et al.*, 1991 found α_1-acid-glycoprotein levels were lower in menstruating women, decreased by estrogen and increased after menopause. This would in turn affect the binding of basic drugs. Small but significantly lower protein binding in women than men of comparable age have been reported for imipramine, chlordiazepoxide, desmethyldiazepam, diazepam and nitrazepam (Wilson, 1984).

Multiple underlying physiologic factors may be responsible for gender differences in amount of plasma proteins and extent of drug binding, thus, the reader is referred to informative reviews on the subject (Lin, *et al.*, 1987; Bonate, 1991; Wilson, 1984). Furthermore, relative proportions of lean body mass are higher in males than females and adipose tissue is generally more developed in females than males which may play a role in tissue distribution of drugs (Giudicelli and Tillement, 1977).

1.1.3. Metabolism

Most of the currently available data regarding sex dependent metabolism results from the study of hepatic metabolism in the rat. At present, two postulates have been formulated to explain sex regulation of xenobiotic metabolizing enzyme activity (Bonate 1991). The first involves

enzyme "imprinting" during the perinatal period where the presence or absence of gonadal hormones results in an "imprinting" of sexual identification, which in turn, determines the ability to respond to androgens thereby influencing enzyme activity in the adult (Bonate, 1991; Skett, 1987). The second postulate involves androgenic maintenance of sex variations in drug metabolizing enzymes in the adult *via* the hypothalamic-pituitary-gonadal axis (Bonate, 1991). The effects of androgens are believed to be largely mediated through sex differences in the phasic nature of growth hormone secretion although somatostatin and insulin levels have also been investigated as a means of explaining gender differences in metabolism (Bonate 1991).

There has been extensive investigation into sex dependent alcohol dehydrogenase and cytochrome P-450 mediated metabolism in both animals and humans (Seitz, *et al.*, 1992; Frezza, *et al.*, 1990; Skett, 1988; Skett, 1987), however, only a small number of drugs have been investigated for gender differences in phase II conjugations in humans (Mulder, 1986). The majority of research completed on gender differences in phase II metabolism has looked at xenobiotic glucuronidation in the rat although sex differences in rat sulfation and glutathione-S-transferase activity have also been reported (Kane and Chen, 1987; Mulder, 1986). Since gender dependent glucuronidation in the rat is most pertinent to our research work, this topic will be introduced in some detail.

In general, male rats may have higher levels of uridine 5' diphosphate glucuronic acid (UDPGA) and activity of uridine 5' diphosphate glucuronosyltransferase (UDPGT) in liver towards various substrates than females (Chhabra and Fouts, 1974; Graham and Skett, 1987; Mulder, 1986; Rush *et. al.*, 1983). In contrast, female rats may show higher activity of uridine 5' diphosphate glucose dehydrogenase, the enzyme that catalyzes formation of UDPGA, however, this is not a rate limiting step in glucuronidation (Muller-Oerlinghausen and Kunzel, 1968). In rats, sex differences in the 24 h urinary excretion of glucuronidated metabolites have been found to correlate with the presence or absence of renal UDPGT activity towards various substrates (Skett, 1987; Rush *et al.*, 1983). It appears for many substrates, that renal glucuronidating activity and subsequent renal excretion of formed glucuronidated metabolites is greater in the female of the species (Ellison *et al.*, 1989)

Sex dependent metabolism of the NSAIDs - piroxicam (Roskos and Boudinot, 1990), diflunisal (Macdonald *et al.*, 1990), tenoxicam (Campanella and Jamali, 1993) and etodolac (Corrigan and Jamali, 1993) has been used, in part, to explain sex related differences in pharmacokinetics of these agents in the rat.

Attempts to identify the specific hormone(s) responsible for gender differences in metabolism have largely centered upon ablation of endocrine organs and/or hormonal treatment in animals (Bertagni *et. al.*, 1972; Blantz

et. al., 1988; Gothoskar and Weisburger, 1980; Skett *et. al.*, 1980; Tanaka *et. al.*, 1992). Reports of the effect of sex hormones on xenobiotic metabolism are often complex or contradictory, thus, the reader is directed to several excellent reviews listing sex variations in specific tissue metabolism of drugs (Skett, 1987, 1988; Mulder, 1986; Giudicelli and Tillement, 1977).

1.1.4. Excretion

Gender appears to play an important role in the CL_r of drugs and regulatory functions of the kidney, namely renal hemodynamics. Serum creatinine levels are generally lower in females than males, irrespective of muscle mass (Nielsen *et al.*, 1973). For drugs excreted solely by the kidneys, elimination $t_{1/2}$ can be related to steady state serum creatinine (Gibaldi and Perrier, 1982), thus, $t_{1/2}$ of such drugs as kanamycin, gentamicin and digoxin are shorter in females (Carraro-Eduardo *et al.*, 1993; Giudicelli and Tillement, 1977). Furthermore, sex-differences in renal metabolism *(discussed under 1.1.3)* can also be a factor in the CL_r of a drug.

Of particular interest are reports of striking gender differences in renal vascular resistance and renal plasma flow in the rat resulting from prostaglandin inhibition by the NSAIDs - indomethacin and mefenamic acid (Munger and Blantz, 1990). Net vasoconstriction in the male rats was observed as is expected due to inhibition of PGE_2 and PGI_2 (both

vasodilatory) by the NSAIDs; however, in female rats, renal vascular resistance unexpectedly decreased after similar doses of the NSAIDs.

Biliary excretion and enterohepatic recirculation of drugs are generally quantitatively more important in animals than humans (Rollins and Klaassen, 1979; Fleck and Braunlich, 1984). An important route of elimination for aryl carboxylic acids such as the NSAIDs is *via* formation of acyl-glucuronidated metabolites in the liver and excretion into bile whereupon they may be recirculated or eliminated in feces (Mayer, 1990). Gender dependent hepatic metabolism or active transport processes at the level of entry into the hepatocyte or excretion out into bile may influence the amount of drug available for biliary excretion and subsequent fecal elimination or reabsorption, thus significantly altering the plasma profile of a drug. Cleavage to the aglycone in the intestine following recirculation may be influenced by microflora, intestinal transit time, luminal contents and bile duct permeability, all of which may differ due to gender (Moseley, 1990). Sex differences in biliary excretion in the rat have been implicated in the dimorphic pharmacokinetics of etodolac (Corrigan and Jamali, 1993), sulfobromophthalein (Kanai *et al.*, 1988) and tartrazine and lissamine fast yellow dye (Bertagni *et al.*, 1972).

1.2. Ketoprofen

Ketoprofen (Orudis™; Rhone-Poulenc-Rorer and Oruvail™; May & Baker

Pharma), a chiral NSAID of the 2-arylpropionic acid (2APA) derivative series, was first synthesized by Farge, Messer and Moutonnier (French Patent 1546478) at the Rhone-Poulenc Research Centre in Paris, France in 1967. Initial research into the biochemical and pharmacokinetic properties of ketoprofen was carried out by the Rhone Poulenc Laboratories research group under leadership of Dr. L. Julou (Fossgreen, 1976). In 1973, ketoprofen was introduced in France and the United Kingdom and following that in North America for the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (Avouac and Teule, 1988). Today, ketoprofen is also used as an analgesic for mild to moderate post-operative, postpartum and chronic cancer pain (Sunshine *et al.*, 1993; Sunshine and Olson, 1988), dental pain (Cailleteau, 1988), and dysmenorrhea (Mehlisch, 1988). Ketoprofen has now been used worldwide for over 20 years and comparative studies with other NSAIDs have found ketoprofen (200-300 mg/day) to be more or equally as efficacious as phenylbutazone (600 mg/day), indomethacin (100-150 mg/kg), ibuprofen (1200 mg/day), piroxicam (20 mg/day), naproxen (500-1000 mg/day), diclofenac (75 mg/day) or aspirin (4 g/day) in the treatment of rheumatoid arthritis (Veys, 1991).

In Canada, ketoprofen is available in a variety of formulations - 50 mg capsules, 50 and 100 mg enteric coated tablets, 200 mg enteric coated sustained release tablets and 50 and 100 mg suppositories (Orudis™,

Rhone-Poulenc-Rorer; Rhodis™, Rhodiapharm), 150 and 200 mg sustained release pellets (Oruvail™; May & Baker, Pharma) as well as generic equivalents of the 50 and 100 mg enteric coated tablets (Apo-Keto-E™; Apotex, Novo-Keto-EC™; Novopharm) (Compendium of Pharmaceuticals and Specialties, 1993). In addition, 100 mg *i.v.* and intramuscular injections as well as a 2.5% topical gel are available in Europe (Veys, 1991; Avouac and Teule, 1988).

The formulations of ketoprofen that are presently marketed are a racemic (rac) mixture of the R and S enantiomers despite antiinflammatory activity residing with only the S-enantiomer (Hayball *et al.*, 1992; Hutt and Caldwell, 1984). However, there seems to be little stereoselectivity in humans as judged by the pharmacokinetics of each enantiomer (Foster *et al.*, 1990; 1988a; 1988b) which will be discussed in detail later.

1.2.1. Chemistry of ketoprofen

The compound (±)-2-(3-benzoylphenyl)-propionic acid; $C_{16}H_{14}O_3$, hereafter known as ketoprofen is a white or slightly colored, odourless, tasteless powder with an irritant dust (Liversidge, 1981). The molecular weight is 254.29; melting point is 93-95 °C and pk_a in methanol:water (3:1) is 5.937 (Liversidge, 1981). The chemical structure of ketoprofen is given in Figure 1.

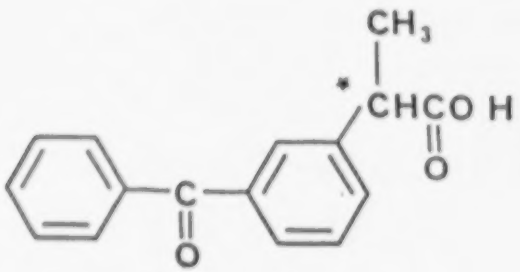

Figure 1. Structure of ketoprofen. Chiral centre is denoted by asterisk (*).

Ketoprofen is soluble in ether, ethanol, acetone, chloroform, ethyl acetate and alkaline solutions, but is only slightly soluble in water (Merck Index, 1983). It is very stable at room temperature and resistant to decomposition even after dissolution in ethyl acetate and storage for several weeks at 4 °C or heating at pH 1 at 98 °C for 30 min (Jefferies *et al.*, 1979). The wavelengths of maximum ultraviolet (UV) absorbance of ketoprofen are 261 nm and 255 nm in aqueous and nonaqueous solutions respectively (Liversidge, 1981).

1.2.2. Analytical Methods

Historically, methods including colorimetric and polarographic analysis, tritium labelling and thin layer or gas chromatography have been used for the

detection of ketoprofen in biological samples (Liversidge, 1981; Fossgreen, 1976). Currently, most of these methods have been replaced by high performance liquid chromatography (HPLC) with UV detection. For a comprehensive listing of nonstereospecific HPLC assays for ketoprofen, the reader is directed to the review of Jamali and Brocks, 1990.

Due to the presence of an asymetric carbon centre (Figure 1), ketoprofen exists as two enantiomers. Both enantiomers have been isolated and assigned absolute configuration (Rendic *et al.*, 1975). Since 1986, a number of stereospecific HPLC methods for the chromatographic separation of ketoprofen enantiomers have been described in the literature. The majority of analytical methods involve precolumn derivatization to form amide diastereomeric derivatives which then are chromatographically separated (Table I). All the assays listed in Table I are reverse-phase methods with the exception of Sallustio *et al.*, 1986 who utilize both reverse and normal phase separation methodology. Other available stereospecific HPLC methods involve use of chiral columns (Shibukawa *et al.*, 1993; Oda *et al.*, 1991; Menzel-Soglowek *et al.*, 1990) or coupled achiral-chiral column separation (Oda *et al.*, 1992; Yagi *et al.*, 1990). In addition, several enantiospecific gas-chromatographic methods are available (Jack *et al.*, 1992; Blessington *et al.*, 1989, Singh *et al.*, 1986).

Table I. Stereospecific high performance liquid chromatographic assays for ketoprofen

Reference	MQC[1]	Volume	Specimen	Derivatizing Reagent
Sallustio *et al.*, 1986	0.2	0.2 mL	Plasma	R-2-phenylethylamine
Bjorkman *et al.*, 1987	0.1	1.0 mL	Plasma	L-leucinamide
Foster *et al.*, 1987,1989a, 1989b	0.05	0.5 mL	Plasma, Urine, Synovial Fluid, Bile	L-leucinamide
Hayball *et al.*, 1991	0.15	1.0 mL	Plasma	S-1-phenylethylamine

[1] MQC = Minimum Quantifiable Concentration reported for each enantiomer (mg/L)

Probenecid produces marked changes in the pharmacokinetics of ketoprofen which may have therapeutic consequences (Upton *et al.*, 1982). As a result, the simultaneous analysis of ketoprofen and probenecid has been investigated. Upton *et al.*, 1980 reported an HPLC method for ketoprofen, naproxen and probenecid that requires the joining of two columns in series and adjustment of mobile phase and flow-rate in order to measure divergent concentrations of the analytes. This assay, however, is not stereospecific.

1.2.3. Basic pharmacology

The derivatives of arachidonic acid metabolism are potent mediators of pain and inflammation in arthropathic conditions. There are two pathways by which these compounds are produced - the action of cyclooxygenase on arachidonic acid leads to formation of prostaglandins and thromboxanes, whereas the action of lipoxygenase leads to formation of leukotrienes and hydroxyeicosatetraenoic acid (Veys, 1991). The primary mechanism of action of ketoprofen and other NSAIDs is the inhibition of cyclooxygenase (prostaglandin synthetase), leading to a decrease in both prostaglandin and thromboxane synthesis (Julou *et al.*, 1976). In addition, ketoprofen inhibits the action of lipoxygenase although there is doubt as to the clinical relevance of the extent of inhibition (Williams and Upton, 1988). The therapeutic usefulness of ketoprofen is thus related to its antiinflammatory,

analgesic and antipyretic properties.

1.2.3.1. Antiinflammatory action

Julou and co-workers completed numerous tests on the antiinflammatory properties of ketoprofen employing carrageenan-induced edema and abscess in the rat, ultraviolet erythema in the guinea pig and adjuvant polyarthritis in the rat (Julou *et al.*, 1976). Inhibition of edema, abscess and erythema became significant at 3, 1.4 and 7.5 mg/kg *p.o.* doses of ketoprofen respectively, thereby showing comparative activity to results seen with indomethacin in the same models. At a daily oral dose of 2.5 mg/kg, ketoprofen and indomethacin offered significant protective activity in rat adjuvant arthritis. There are numerous reports of the clinical antiinflammatory efficacy of ketoprofen in humans (Veys, 1991; Williams and Upton, 1988; Fossgreen , 1976)

1.2.3.2. Analgesic action

Analgesic properties of ketoprofen have been investigated using the writhing syndrome induced in mice by the *i.p.* injection of phenylbenzoquinone or bradykinin and the paw pressure test in the rat (Julou *et al.*, 1976). Strong analgesic activity that was not significantly different from that of indomethacin was observed with ketoprofen. In clinical trials, the analgesic effect has been repeatedly demonstrated in

humans. Single 100 mg oral doses of ketoprofen have been found to be superior to 650 mg acetaminophen and placebo and at least as effective as the combination of 650 mg acetaminophen and 10 mg oxycodone for treatment of severe postoperative pain after cesarean section (Sunshine *et al.*, 1993). In addition, ketoprofen analgesia has been found effective in postpartum, general surgery and chronic cancer pain (Sunshine and Olson, 1988) and primary dysmenorrhea (Mehlisch, 1988).

1.2.3.3. Antipyretic action

Ketoprofen has some antipyretic activity, and has been found to be 3-4 times as effective as indomethacin against hyperthermia induced in the rat by brewer's yeast suspension and in the rabbit by injection of antigonoccocal vaccine (Julou *et al.*, 1976).

1.2.3.4. Other properties

Other properties of ketoprofen may contribute to its therapeutic action - inhibition of bradykinin, stabilization of lysosomal membranes against osmotic damage and prevention of the release of lysosomal enzymes that cause tissue destruction during the inflammatory process (Veys, 1991). In addition, ketoprofen resulted in only a "modest" reduction in proteoglycan synthesis compared with indomethacin, diclofenac, tiaprofenic acid and sodium pentosan polysulphate (Collier and Ghosh, 1989) in an *in vitro*

experiment in the rabbit. In an *in vitro* experiment utilizing human normal young, adult and osteoarthritic cartilage, it was found that ketoprofen added to the culture increased cartilage synthesis at a concentration of 10^{-4} M in normal young cartilage but had no effect on the latter two (Wilbrink *et al.*, 1991).

1.2.3.5. Enantiospecific activity

Early studies on the enantiomers of the 2APA NSAID class, to which ketoprofen belongs, established that antiinflammatory activity resides primarily in the S-enantiomer (Hutt and Caldwell, 1984). Hayball *et al.*, 1992 assessed the antiplatelet effect of ketoprofen by measuring the inhibition of platelet thromboxane B_2 generation during controlled clotting of whole blood from healthy human volunteers. S-ketoprofen was solely active in inhibiting its production whereas R-ketoprofen was devoid of activity but did not affect the potency of its S-antipode. A recent report of the enantiospecific action of ketoprofen describes a dose dependent action of S-ketoprofen in the prevention of immobilization (tetonomy) induced bone loss in weanling female rats (Zeng, *et al.*, 1993).

1.2.4. Pharmacokinetic studies in humans

In humans, ketoprofen exhibits only limited enantioselectivity in its disposition and no interaction between enantiomers. It is estimated that ≈

9-12% metabolic bioinversion of the R- to S-enantiomer occurs in humans (Jamali *et al.*, 1990). In light of this, data generated from nonstereospecific analysis may generally be used to explain the pharmacokinetics of the individual enantiomers in humans. A brief review of the absorption, distribution, metabolism and excretion of ketoprofen in humans follows.

1.2.4.1. Absorption

There are various oral preparations (regular release, sustained-release and enteric-coated) of ketoprofen available on the market. Following oral administration of a regular release formulation, there is rapid and virtually complete absorption (absolute bioavailability estimated to be $\geq$ 92%) with peak concentrations (C_{max}) occurring between 0.5 and 2 h post-dose (t_{max}) (Jamali and Brocks, 1990). There does not appear to be any enantioselectivity in absorption of ketoprofen and absorption characteristics following multiple doses are similar to those seen with single doses (Foster *et al.*, 1988). An excellent listing of the oral absorption characteristics of ketoprofen can be found elsewhere (Jamali and Brocks, 1990).

The rate of absorption of ketoprofen is slowed in the various sustained-release preparations resulting in longer t_{max} and lower C_{max} than regular release, however AUCs are comparable (Houghton *et al.*, 1984; Borsa *et al.*, 1983; Flouvat *et al.*, 1983). The presence of food may delay the rate of ketoprofen absorption but not the extent (Bannwarth *et al.*, 1988).

Absorption may show circadian variations. In one study it was found that C_{max} was twice as high when ketoprofen was administered at 0700 than at other times in 8 healthy human volunteers (Ollagnier *et al.*, 1987). Topical preparations show limited systemic absorption (Flouvat *et al.*, 1989) whereas intramuscular and rectal administration of ketoprofen result in significant systemic absorption (Ishizaki *et al.*, 1980).

1.2.4.2. Distribution

Similar to other 2APA NSAIDs, ketoprofen is extensively (> 95%) bound to plasma albumin and as a result is primarily confined to the plasma compartment, evidenced by its small apparent volume of distribution in humans (V_d = 0.1 to 0.2 L/kg) (Jamali and Brocks, 1990). Stereoselective protein binding does not seem to play a role in the *in vivo* disposition of ketoprofen in humans; however, the stereoselectivity of *in vitro* protein binding of ketoprofen has been an issue of some debate. Rendic *et al.*, 1980, using individual enantiomers and solutions of protein prepared from crystalline human serum albumin found that at low concentrations of ligand and protein, R-ketoprofen is bound more extensively than S-ketoprofen, although this enantioselectivity disappeared at higher concentrations. Similarly, Dubois *et al.*, 1993a report stereoselective binding but used concentrations of ketoprofen far exceeding therapeutic levels and solutions of lyophilised human serum albumin to simulate plasma and synovial fluid.

Hayball *et al.*, 1991 using therapeutic concentrations of ketoprofen enantiomers and human plasma reported no stereoselectivity in binding.

There is also some evidence of irreversible binding of ketoprofen glucuronide conjugates to plasma albumin following hydrolysis, intramolecular rearrangement and binding. This phenomenon has been investigated both nonstereospecifically (Dubois *et al.*, 1993b) and stereospecifically (Hayball *et al.*, 1992). In both instances albumin-catalyzed hydrolysis of the conjugate was reported and a proposed stereoselectivity in binding to plasma proteins was suggested in the latter study.

Ketoprofen does penetrate the synovium and substantial concentrations have been found in synovial fluid, which is the proposed site of action for NSAIDs (Wallis and Simkin, 1983). The C_{max} in synovial fluid is lower and occurs later than plasma and disposition into this site does not appear to be stereoselective (Foster *et al.*, 1989).

1.2.4.3. Metabolism

The major metabolite of ketoprofen in humans is depicted in Figure 2. Ketoprofen is extensively metabolized to a water soluble acyl glucuronide conjugate which is excreted in the urine in humans (Jamali and Brocks, 1990; Sallustio *et al.*, 1988; Houghton *et al.* 1984). Renal elimination of ketoprofen glucuronides is compromised in patients with renal dysfunction (Hayball *et al.*, 1993; Stafanger *et al.*, 1981). Substantial concentrations of

glucuronidated enantiomers (S > R) in plasma of elderly arthritic patients have been reported whereas glucuronide conjugates are not detectable in plasma of healthy young or young arthritic patients (Foster *et al.*, 1988).

There may be evidence of non-conjugated metabolites of ketoprofen, possibly hydroxylated products, however such metabolites have not been isolated to date and appear to be very species dependent (Delbarre, 1976; Populaire *et al.*, 1973).

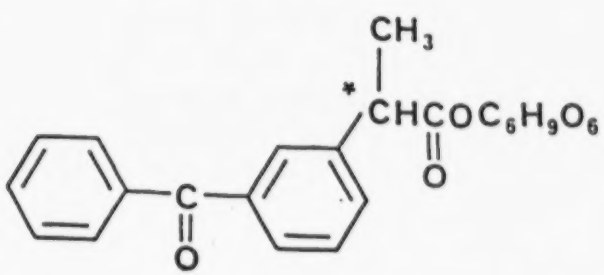

Figure 2. The major metabolite of ketoprofen in humans and rats. Chiral centre denoted by asterisk (*).

The R-enantiomer of numerous chiral 2APA NSAIDs is known to undergo metabolic bioinversion to the active S-antipode; however, this pathway is highly drug and species dependent (Jamali, 1988; Hutt and Caldwell, 1983). In healthy human females, only 9-12% of an administered dose of R-ketoprofen was inverted to active S-ketoprofen (Jamali *et al.*, 1990).

1.2.4.4. Excretion

The terminal elimination $t_{1/2}$ of ketoprofen in humans is reported to be in the range of 1.5 to 4.0 h (Jamali and Brocks, 1990). Ketoprofen is rapidly and extensively eliminated in urine as a glucuronide metabolite (≈50-90% of the administered dose) (Jamali *et al.*, 1990; Foster *et al.*, 1988; Houghton *et al.*, 1984). Negligible quantities of parent drug are found in urine (Foster *et al*, 1988; Upton *et al.*, 1980).

The cumulative urinary excretion of glucuronidated ketoprofen in humans is stereoselective in favor of the S-enantiomer. Foster *et al.*, (1988a) reported the mean S:R ratio in urine to be 1.2 which was explained by the limited chiral inversion or preferential biliary excretion of R-ketoprofen. The latter was found unlikely as in subsequent work, biliary elimination of ketoprofen was found to be minimal in humans, with only 1.0% of S- and 0.8% of R-ketoprofen recovered in the bile of cholecystectomised patients (Foster *et al.*, 1989). Similarly, only 4.1% of a dose of tritiated ketoprofen was recovered in feces of healthy subjects (Delbarre, 1976). Enterohepatic recirculation of ketoprofen in humans appears to be negligible.

An excellent linear correlation ($r^2 = 0.90$) exists between rac-ketoprofen dose (50 mg to 200 mg) and AUC indicating elimination of the enantiomers is not saturable over the normal therapeutic dosage range (Jamali and Brocks, 1990). The S:R concentration ratios were not affected by increasing doses thus the lack of stereoselectivity holds with normal

therapeutic doses.

1.2.5. Pharmacokinetic studies in animals

In contrast to the lack of stereoselectivity in humans, the pharmacokinetics of ketoprofen display marked stereoselectivity in rabbits (Abas and Meffin, 1987), horses (Jaussaud *et al.*, 1993) and rats (Foster and Jamali, 1988; Iwakawa *et al.*, 1991).

1.2.5.1. Rats

To date, the majority of pharmacokinetic studies of NSAIDs have used male rats as animal models in order to avoid the confounding factor of the female oestrous cycle. Male rats have historically been used for studies of ketoprofen (Satterwhite and Boudinot, 1992; Iwakawa *et al.*, 1991; Foster *et al.*, 1988) despite findings of no evidence of pharmacokinetic changes of ketoprofen during the oestrous cycle in female rats (Bruguerolle and Bovenot, 1991).

The stereoselective pharmacokinetics of ketoprofen in the rat have been studied following administration of 10 mg/kg *oral, i.p.* and *i.v.* doses of rac-ketoprofen to intact and bile-duct cannulated male Sprague Dawley rats (Foster and Jamali, 1988). Results indicate marked stereoselective disposition and the mean pharmacokinetic parameters from that study are summarized in Table II. The extent of inversion of R- to S-ketoprofen is

estimated to be > 56% after administration of racemate with substantial presystemic inversion occurring in the rat. Iwakawa *et al.*, 1991 found chiral inversion of ketoprofen to be in the order of 79% in male rats after dosing with individual enantiomers. Contrary to human data (Foster *et al.*, 1989), there is extensive stereoselective elimination of the drug through bile in male rats and low recovery of the dose in urine, irrespective of route of administration (Table II). Similar biliary excretion in the male rat has been reported by Menzel *et al.*, 1993.

1.2.5.2. Other animal species

There are a limited number of stereospecific studies of ketoprofen pharmacokinetics utilizing animal models other than rats. The enantioselective disposition of ketoprofen has been studied in male rabbits where 9% of R-ketoprofen was found to invert to its S-antipode (Abas and Meffin, 1987). Enantioselective disposition has also been described in the horse (Jassaud *et al.*, 1993).

1.2.6. Side effects and toxicity

Reports of adverse drug reactions to NSAIDs from the years 1968 to 1981 in the United Kingdom revealed ketoprofen had a frequency of side effects comparable with those of naproxen (Spiers, 1988). Ketoprofen demonstrates a reliable efficacy/safety ratio based on drug surveillance data

Table II. Pharmacokinetic indices of ketoprofen enantiomers after various routes of administration to male rats.[1] Data are presented as the mean ± (SD)

Route	n	$AUC_{(0-\infty)}$ (mg.h/L)			$t_{1/2}$ (h)		V_d (L)		% Dose in Urine[2]		
		S	R	S:R	S	R	S	R	S	R	S:R
i.v.	5	811 (391)	80.5 (20.9)	11.8 (9.93)	12.4 (2.63)	19.7 (8.57)	0.11 (0.09)	0.69 (0.23)	9.5 (5.8)	1.6 (0.84)	5.8 (3.0)
oral	5	406 (73.4)	12.9 (3.96)	33.7 (11.2)	nc	nc	nc	nc	5.4 (3.0)	0.85 (0.33)	6.5 (3.0)
i.p.	4	590 (270)	55.9 (35.0)	11.0 (2.64)	11.2 (1.63)	17.2 (14.3)	0.10 (0.05)	0.82 (0.45)	10 (4.8)	1.2 (0.40)	8.7 (2.5)
i.v. (bile-duct cannula)									% Dose in Bile[2]		
	4	85.4 (58.6)	22.8 (18.4)	3.93 (0.70)	0.83 (0.21)	0.65 (0.17)	0.04 (0.02)	0.09 (0.06)	77.9 (3.77)	11.4 (2.48)	7.08 (1.54)

[1] Data is from the study of Foster and Jamali, 1988
[2] As glucuronide conjugate
nc = value not calculated due to insufficient data points

from Europe and the United Kingdom where the drug has been on the market the longest (Avouac and Teule, 1988). Gastrointestinal complications are the most frequent of all serious adverse effects, although renal, hepatic, hematologic and toxic cutaneous reactions have been reported (Veys, 1991; Avouac and Teule, 1988).

An LD_{50} of 100-200 mg/kg of ketoprofen in mice, rats, guinea pigs, rabbits and dogs has been reported and both acute and subacute studies have been performed in these species (Julou *et al.*, 1976). Acute toxicity of ketoprofen was found to be distinctly less than for indomethacin and was marked by sedation, adynamia, diarrhea and emesis with gastrointestinal lesions found at autopsy. Subacute studies revealed gastrointestinal toxicities in rat and dog with rat also displaying renal toxicities (Julou *et al.*, 1976).

There have been numerous studies demonstrating that NSAIDs increase small intestine permeability in humans and animals using urinary excretion of radiolabelled ^{51}Cr-EDTA as a marker (Bjarnason *et al.*, 1984; Wright *et al.*, 1993). A significant dose-related increase in intestinal permeability of ^{51}Cr-EDTA following oral doses of rac-ketoprofen or the individual enantiomers has been reported in male rats (Wright *et al.*, 1993). A linear increase in ^{51}Cr-EDTA permeability following increasing dose of R-ketoprofen despite a constant dose of the S-antipode ($r = 0.986$, $p < 0.05$) suggests that gastrointestinal toxicity of NSAIDs such as ketoprofen may reside with both

R and S enantiomers.

Sex differences in renal vascular resistance and renal plasma flow in the rat arising from prostaglandin inhibition have been reported (Munger and Blantz, 1990) and are discussed under 1.1.4. Excretion. The implications to gender differences in renal toxicity of the NSAIDs such as ketoprofen and if toxicity resides with both enantiomers remain unknown.

Teratogenicity studies in mouse, rat and rabbit revealed lack of any embryonic effects except for slight effects in rabbits receiving high doses of ketoprofen that was attributed to general decline of the condition of the rabbits rather than the drug. Carcinogenicity and mutagenicity studies were negative (Julou *et al.*, 1976).

1.2.7. Drug interactions

A number of clinically significant drug interactions with ketoprofen in humans have been reported and reviewed (Veys, 1991). The most clinically relevant and related to pharmacokinetic properties include interactions with sucralfate (Caille *et al.*, 1987), aspirin (Williams *et al.*, 1981), methotrexate (Thyss *et al.*, 1986), lithium (Singer *et al.*, 1981) and probenecid (Upton *et al.*, 1982; Foster *et al.*, 1989).

1.3. Rationale for study of gender differences in the stereoselective pharmacokinetics of ketoprofen in rats

Historically, pharmacokinetic studies of many drugs including ketoprofen and other NSAIDs have been done primarily in the male of the species. An analysis of papers published in the European Journal of Clinical Pharmacology during the period of 1978-1982 found that more than one-half of all the investigations employed an all male panel of male volunteers (Bonate, 1991). In studies that did employ female subjects, there was a tendency to lump them into one study group and treat them as homogenous in all respects.

In light of marked physiologic differences between males and females it is reasonable to assume there to be marked differences in the disposition of drugs. It may even be possible that the large interindividual variability in diposition and response to NSAIDs may, in part, be explained by consideration of gender. Females are more prone to developing various arthropathic conditions than males and as a result are more extensive users of NSAIDs. Not suprisingly, females are reported to suffer the most side effects, primarily those over 65 years of age, thus, gender dependent disposition is a consideration that warrants exploration (Zeidler, 1991).

We sought to develop an animal model for the study of gender differences in the disposition of chiral NSAIDs. For this purpose we studied the pharmacokinetics of ketoprofen, an NSAID previously known to display

marked stereoselectivity in the rat. We chose the rat as a model due to our early work utilizing this species in adjuvant arthritis and as a result of our preliminary experiments revealing a marked dimorphism in the urinary excretion of ketoprofen which suggested the female rat may be a better animal model for the study of these agents.

It is important to consider the stereoselective nature of the pharmacokinetics of NSAIDs such as ketoprofen for the following reasons. It is known that enantiomers possess the same physical-chemical properties and activity in achiral environments, however, when in a chiral environment, their pharmacologic and pharmacokinetic properties may differ significantly. The chiral nature of biological systems such as the human body stem from chiral macromolecules such as proteins. It follows that the interaction of chiral drugs such as NSAIDs with chiral plasma, transport, metabolic or receptor proteins may be more favourable for one enantiomer than its corresponding antipode. The result may be substrate stereospecificity whereby only one enantiomer interacts with a system and produces an effect or stereoselectivity where there is a preferential interaction of one enantiomer over another, although both have the ability to interact with the system. As a result, any factors involved in drug disposition such as absorption, distribution, metabolism or excretion may be regulated by chiral recognition. Currently, the vast majority of chiral drugs in use continue to be marketed and administered as the racemate: 50:50 portions of R and S

enantiomers, in spite of known differences in activities between enantiomers. Nonstereospecific methodology continues to be used which results in no differentiation of plasma concentrations or pharmacokinetic parameters between enantiomers but rather reflects the sum of the active and less active forms. The interpretation of pharmacokinetic data, in relation to pharmacodynamic properties is rendered misleading or meaningless (Ariens, 1984; Evans *et al.*, 1988; Jamali *et al.*, 1989). For further discussion of chirality, the reader is referred to a number of reviews on stereoselectivity (Caldwell, 1992; Jamali *et al.*, 1989, Tucker and Lennard, 1990).

1.4 Hypotheses

1. A gender dimorphism in the stereoselective pharmacokinetics of ketoprofen in the rat exists and is due to gender differences in:
    a) Renal or biliary clearance
    b) Hepatic or renal glucuronidation
    c) Plasma protein binding
2. Gender differences in elimination of ketoprofen are under control of the sex hormones and can be altered by gonadectomy and concomitant sex hormone treatment.
3. Gender differences in the pharmacokinetics of ketoprofen may result in differences in intestinal toxicity in the rat.

4. Probenecid may be an effective tool to investigate the mechanism of the gender dimorphism of ketoprofen enantiomer pharmacokinetics in the rat.

1.5. Specific objectives and their rationale

1.5.1. Study the gender dimorphism in pharmacokinetics of ketoprofen enantiomers in rats

During preliminary experiments, it was serendipitously discovered that a marked gender difference in urinary recovery of S-ketoprofen glucuronide exists in the Sprague-Dawley rat strain. Hence, we sought to compare the pharmacokinetics of ketoprofen enantiomers between male and female rats by administering rac-ketoprofen to intact and bile-duct cannulated rats in an attempt to elucidate the mechanism of the gender difference. We also performed experiments to:

1.5.1.1. Determine gender and tissue differences in glucuronidation of ketoprofen enantiomers

In the male rat, hepatic glucuronidation of various substrates is generally reported to exceed that of the female rat, whereas renal activity in the female is generally higher than in the male. Consequently, sex differences in liver and kidney tissue glucuronidation of ketoprofen were investigated as potential mechanism(s) contributing to the gender difference in ketoprofen

enantiomer disposition utilizing microsomal preparations of these tissues.

1.5.1.2. Determine effect of gonadectomy and sex hormones

The effects of gonadectomy and/or concomitant sex hormone treatment to alter male and female elimination patterns of drugs have been amply described in the literature. To determine if the process(es) governing the gender dimorphism in ketoprofen disposition is/are under control of the sex hormones, male and female rats underwent gonadectomy with or without concomitant estradiol or testosterone treatment. This was done to ascertain if renal excretion of S-ketoprofen glucuronide was indeed under control of these sex hormones. A review of the literature was done to determine appropriate doses of estradiol and testosterone to use for this experiment.

1.5.1.3. Determine gender differences in plasma protein binding

The clearance of ketoprofen is very dependent on binding to plasma albumin as is often the nature of low extracted, highly protein bound drugs like the NSAIDs (Lin *et al.*, 1987). As a result, gender differences in the *in vitro* binding of ketoprofen enantiomers were investigated as a contributing factor to the sex differences in disposition of ketoprofen in the rat.

1.5.1.4. Determine gender differences in increased permeability of ^{51}Cr-EDTA as a marker of intestinal toxicity

In light of the different disposition profiles, it is plausible that gastrointestinal toxicities of NSAIDs differ between male and female rats, and may be determined, in part, by the relative contribution of urinary or biliary excretion and enterohepatic recirculation. The possibility of the contribution of a sex difference in biliary excretion of S-ketoprofen and its consequence to the ketoprofen induced increase in intestinal permeability in the rat was measured by urinary recovery of ^{51}Cr-EDTA.

1.5.2. Study effect of probenecid on the stereoselective pharmacokinetics of ketoprofen in male and female rats

Probenecid is reported to interact with ketoprofen at the level of excretion (Upton *et al.*, 1982), thus, we sought to determine if the extent of interaction has a gender component due to the marked sex differences in disposition of ketoprofen in the rat. An elevation of the plasma concentrations of active S-ketoprofen due to probenecid may have therapeutic consequences, as prior studies in our laboratory utlilizing the adjuvant arthritic rat model have shown a relationship between concentration of S-ketoprofen and antiinflammatory efficacy (Jamali and Brocks, 1990). We completed experiments to:

1.5.2.1. Establish a suitable stereospecific method for simultaneous quantification of ketoprofen enantiomers and probenecid in biological samples

In order to investigate the interaction we were required to develop an HPLC assay to simultaneously quantitate ketoprofen and probenecid. A previously reported method for ketoprofen alone was unsuitable due to the interference of probenecid and the internal standard peaks and non-linearity attributable to extraction solvent volume.

1.5.2.2. Determine the stereoselectivity and concentration dependence of the interaction of probenecid and ketoprofen enantiomers

Previous work into interactions of probenecid with NSAIDs have failed to simultaneously quantitate probenecid concentrations, thus, it was not known whether the interaction was either enantioselective or probenecid concentration dependent.

1.5.2.3. Determine effect of probenecid on the disposition of ketoprofen enantiomer glucuronide conjugates

In an attempt to differentiate between the competitive effect of probenecid on formation of ketoprofen enantiomer glucuronides and elimination of formed glucuronides, we studied the effect of probenecid on *i.v.* doses of R- and S-ketoprofen glucuronides in both intact and bile-duct

cannulated rats.

1.5.2.4. Determine effect of probenecid on glucuronidation of ketoprofen enantiomers

Probenecid is reported to competitively inhibit glucuronidation and compete for biliary/renal excretion of formed glucuronides of NSAIDs. A 'futile cycle' where, following enterohepatic recirculation, the glucuronide hydrolyzes liberating parent drug which can once more be glucuronidated and recirculated and so on may be begun. The competitive effect of various probenecid concentrations on the glucuronidation of ketoprofen enantiomers was investigated utilizing hepatic and renal rat microsomal preparations.

2. EXPERIMENTAL

2.1. Chemicals

Ketoprofen; *(±)-2-(3-benzoylphenyl)-propionic acid*, the internal standard, indoprofen; *(±)-p-(1-oxo-2-isoindolinyl)- hydratropic acid* and probenecid; *p-(dipropylsulfamyl)-benzoic acid,* were purchased from Sigma Chemical Co. (St. Louis, MO). In addition, kits employed for determination of albumin and total microsomal protein concentrations, all reagents and chemicals used for the microsomal incubations, as well as urethane and L-leucinamide hydrochloride were also purchased from Sigma Chemical Co. ^{51}Cr-EDTA specific activity (570 MCi/mg) was purchased from Dupont NEN (Wilmington, DE). Estradiol valerate *(Delestrogen™)* was purchased from Squibb (Montreal, QC) and testosterone cypionate *(DepoTestosterone™)* was purchased from Upjohn (Don Mills, ON).

Ethyl chloroformate, isooctane, chloroform, HPLC grade water, H_2SO_4, NaOH, NaH_2PO_4, Na_2HPO_4, $MgCl_2$ and diethyl ether were purchased from B.D.H. Chemicals (Edmonton, AB). Methoxyflurane (*Metofane™*) was purchased from Pittman-Moore, Ltd. (Mississauga, ON), polyethylene glycol (PEG) 400 from Fisher Scientific Ltd. (Edmonton, AB) and heparin (*Hepalean™*) from Organon Teknika (Toronto, ON). Any other solvents or chemicals employed were of analytical grade.

2.2. Assays

2.2.1. Apparatus and chromatographic conditions

The HPLC system (Waters Scientific, Mississauga, ON) consisted of a Model M-45 pump, Model 481 UV spectrophotometer, Model 712 WISP automatic sample processor and a Hewlett-Packard (Avondale, PA) Model 3390A integrator-recorder operated at ambient temperature. A 10 cm stainless steel reversed phase column (4.6 mm-*i.d.*) octadecyl-bonded silica (5 μm Partisil 5 ODS-3, Phenomenex, Torrance, CA) attached to a 5 cm guard column (*i.d.* 5 mm) packed with 37-53 μm C_{18} material was used. The mobile phase, unless otherwise stated, consisted of 0.06 M KH_2PO_4-acetonitrile-triethylamine (65:35:0.1) pumped at a flow-rate of 1 mL/min. The detector wavelength was 275 nm. The peak area method (ketoprofen enantiomer or probenecid/internal standard) was used to calculate response.

2.2.2. Standard solutions

Stock solutions and dilutions of rac-ketoprofen, probenecid and rac-indoprofen were prepared in 0.01 M NaOH in HPLC grade water.

2.2.3. Sample preparation and analysis

2.2.3.1. Ketoprofen assay

A stereospecific assay for ketoprofen, previously developed in our

laboratory (Foster and Jamali, 1987) was used for analyzing samples arising from experiments where only ketoprofen was administered to rats. For convenience, the assay was modified slightly by using indoprofen rather than fenoprofen as an internal standard and the mobile phase was changed to 0.06M KH_2PO_4-acetonitrile-triethylamine (65:35:0.1). All other conditions remained the same.

2.2.3.2. Ketoprofen and probenecid assay

The assay of Jamali and Foster, (1987) was further modified and validated as follows in order to analyze samples arising from experiments where both rac-ketoprofen and probenecid were administered to rats. To 100 μL rat plasma samples containing ketoprofen and probenecid was added 100 μL of an aqueous solution of 100 μg/mL rac-indoprofen as internal standard. To acidify, 100 μL of 0.6 M H_2SO_4 was added. The sample was extracted with 5 mL of a mixture of isooctane:isopropanol (95:5) after vortex mixing for 30 s and centrifuging on a Clay-Adams centrifuge for 5 min at 1800 x ġ. The organic layer was transferred to clean glass tubes and 5 mL of HPLC grade water added. Samples were again vortex mixed (30 s) and centrifuged (3 min). The organic layer was aspirated off and 200 μL of 0.6 M H_2SO_4 added to the remaining aqueous layer. Five mL of chloroform was added and the samples once again vortex mixed (30 s) and centrifuged (3 min). The aqueous layer was aspirated off and the organic phase

evaporated to dryness (SVC100H Savant Speed Vac Concentrator and Refrigerated Condensation Trap, Emerston Instruments, Scarborough, ON, Canada). The residue was reconstituted with 100 μL of 50 mM triethylamine in acetonitrile while vortex mixing for 30 s. 50 μL of 60 mM ethyl chloroformate in acetonitrile and 50 μL of a solution of 1 M L-leucinamide hydrochloride and 1 M triethylamine in methanol were added at 30 s intervals. After 2 min, 50 μL of HPLC grade water was added. Aliquots of 10-60 μL of the resulting solutions were injected into the HPLC system.

The glucuronidated ketoprofen enantiomers in 100 μL urine samples were analyzed by the addition of 50 μL of 1 M NaOH to the sample in order to bring about alkaline hydrolysis of the conjugates. The samples were subsequently acidified with 100 μL of 0.6 M H_2SO_4 in excess of the volume of 1 M NaOH used for hydrolysis. Sample preparation was continued as for plasma. The amount of glucuronidated drug is taken to be the difference of the hydrolyzed and unhydrolyzed sample.

2.2.3.2.1. Extraction yields

The extraction efficiency was evaluated by extracting rac-ketoprofen and probenecid (10 μg/mL) from 100 μL spiked rat plasma and urine samples (n = 3) in the absence of internal standard according to the procedure detailed for sample preparation. Exact volumes (4 mL) of each layer were

used for each subsequent step in the sample preparation to insure accuracy in determining yield. The residue obtained after evaporation of the chloroform layer was reconstituted with 200 μL of methanol with vortex mixing for 30 s and 100 μL of internal standard added. Aliquots of 20 μL of the resulting solutions were chromatographed nonstereospecifically using the exact conditions reported for sample analysis with a mobile phase of 0.06 M KH_2PO_4-acetonitrile -triethylamine (75:25:0.1). The eluted peak area ratios (ketoprofen enantiomer or probenecid/internal standard) were compared with the ratios obtained after direct injection of unextracted samples of equivalent concentrations of the drugs (n = 6) serving as reference.

2.2.3.2.2. Derivatization yields

To investigate the overall derivatization reaction, 100 μL rat plasma and urine samples (n = 3) were spiked with 100 μg/mL of rac-ketoprofen, probenecid and rac-indoprofen and analyzed in accordance with the reported procedure using the mobile phase described in *2.2.3.2.1* for extraction yield. The derivatization yield was calculated by comparing the peak areas of the derivatized ketoprofen and indoprofen diastereomers and probenecid with the corresponding underivatized rac-ketoprofen, rac-indoprofen and probenecid peak areas in the same chromatograms.

2.2.3.2.3. Standard curves and accuracy

The ketoprofen enantiomers and probenecid were quantified against standard curves obtained from sets of solutions prepared by spiking 100 μL of blank rat plasma and urine with rac-ketoprofen and probenecid. The spiked samples provided individual ketoprofen enantiomer and probenecid concentrations of 1, 10, and 40 μg/mL when analyzed by this method. Linearity was investigated by the analysis of standardized samples ranging in concentration from 0.5 μg/mL to 100 μg/mL. Samples containing 1, 10 and 40 μg/mL ketoprofen enantiomers in the absence of probenecid and 1, 10 and 40 μg/mL of probenecid in the absence of ketoprofen were analyzed in order to compare the similarity of results obtained in the presence and absence of either drug. The precision of the assay was determined by the analysis of three sets of three standard sample concentrations daily to determine intraday variability over five separate days to assess interday variability. The accuracy was investigated by the comparison of measured to theoretical concentrations.

2.2.3.2.4. Other compounds

Rac-indoprofen, rac-carprofen, rac-flurbiprofen, piroxicam, clofibric acid (Sigma, St. Louis, MO), rac-fenoprofen (Eli Lilly, Toronto, ON), rac-cicloprofen (Squibb, Princeton, NJ), rac-pirprofen (Ciba-Geigy, Mississauga, ON), R-ibuprofen (Sepracor, Marlborough, MA), S-ibuprofen (Ethyl Chemicals

Group, Baton Rouge, LA), rac-etodolac (Ayerst, Montreal, QC), S-naproxen (Syntex, Palo Alto, CA) and rac-tiaprofenic acid (Roussel, Montreal, QC) were also subjected to this analysis method in order to determine a suitable internal standard for use with ketoprofen enantiomers and probenecid.

2.3. *In vivo* studies in rats

2.3.1. Surgical methods

2.3.1.1. Jugular vein cannulation

Adult age matched female and male Sprague Dawley rats were used for all studies. Catheterization of the jugular vein was completed under methoxyflurane anesthesia (*Metofane*^{TM}, Pitman-Moore Ltd., Mississauga, ON) with silastic tubing (0.58 mm *i.d.* x 0.965 mm *o.d.*; Clay Adams, Parsippany, NJ). The animals were allowed to recover overnight with food and water *ad libitum*. The next morning and for duration of the studies, rats were individually housed in plastic metabolic cages.

2.3.1.2. Bile-duct cannulation

In addition to jugular vein cannulation according to the procedure described in *2.3.1.1.*, female and male rats were also catheterized with polyethylene tubing (Clay Adams, Parsippany, NJ) at the common bile-duct. The bile-duct cannulated rats were fitted with customized jackets to maintain the biliary catheter in order to facilitate bile collection over the

collection period.

2.3.1.3. Ovarectomy

All gonadectomy surgeries were done under methoxyflurane anesthesia (*Metofane*^{TM}, Pitman-Moore Ltd., Mississauga, ON) following pre-operative cleansing (after shaving) with chlorhexidine gluconate 4% (*Hibitane*^{TM}; Ayerst, Montreal, QC) and povidone iodine 7.5% (*Betadine*^{TM}; Purdue-Frederick, Inc., Toronto, ON). In female rats, bilateral ovarectomy was done *via* small midline dorsal incisions with ovaries severed at the junction of fallopian tube and uterine horn and periovarian fat and blood vessels following ligation (Waynforth, 1980). The muscular layer was sutured and the skin clipped with 9 mm wound clips (*Autoclips*^{TM}; Clay Adams, Parisippanny, NJ) which were removed after 3 days. In sham operated animals, both gonads were identified by surgical exploration without removal.

2.3.1.4. Castration

In male rats, castration was done *via* a small median incision through the scrotal skin and then testicular sacs. The testis accompanied by the vas deferens and spermatic blood vessels were severed distal to a single ligature and removed (Waynforth, 1980). The remaining incisions were closed using *Nexaband*^{TM} Liquid (Tri-Pont Medical, Raleigh, NC). In sham operated

animals, both gonads were identified by surgical exploration without removal.

2.3.2. Drug administration and sample collection

The term *'intact'* refers to male and female rats that have only undergone cannulation at the jugular vein, whereas *'bile-duct cannulated'* refers to rats having undergone cannulation at both the jugular vein and the common bile-duct. A summary of the sample size and the mean weight of rats used in each *in vivo* rat study of the research project is given in **Table III.**

2.3.2.1. Pharmacokinetics in female and male rats

2.3.2.1.1. Intact rats

Rac-ketoprofen (10 mg/kg) in polyethylene glycol (PEG) 400 was administered as a bolus *i.v.* dose into the jugular vein cannula of female and male rats (n = 10 each group). Blood (0.2 mL) was collected from the jugular vein cannula at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 9.0, 12.0 and 24.0 h after the ketoprofen dose. The catheter was flushed with heparin (100 U/mL) after dosing and between each blood sample collection. Blood was centrifuged and the plasma portion separated. Urine was collected from 0-24 h after the ketoprofen dose into containers previously rinsed with 1M HCl to prevent spontaneous hydrolysis of the glucuronide

conjugate. All samples were immediately frozen at -20 °C until analysis.

2.3.2.1.2. Bile-duct cannulated rats

In bile-duct cannulated male and female rats (n = 3 each), plasma was collected at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 9.0 h and bile and urine were collected from 0-9 h after a 10 mg/kg *i.v.* bolus dose of rac-ketoprofen in PEG 400. Bile and urine were collected and stored in containers previously rinsed with 1 M HCl. All specimens were immediately frozen at -20 °C until analysis.

2.3.2.2. Gonadectomy and sex hormones

2.3.2.2.1. Ketoprofen

Female rats were divided into the following treatment groups (n = 4 each): control, ovarectomized and ovarectomized + testosterone treatments. Male rats were also divided into groups (n = 4 each): control, castrated and castrated + estradiol treatments. Rac-ketoprofen (10 mg/kg) dissolved in PEG 400 was administered by the *i.p.* route once a week on the same day for 4 weeks and then again at 11 weeks. Following the previous experiments, sham-operated male and female rats, ovarectomized + estradiol treated female rats and castrated + testosterone treated male rats (n = 4 each) were dosed with rac-ketoprofen at weeks 1 and 4. Rats were individually housed in plastic metabolic cages with food and water *ad libitum*

Table III. Sample size and mean ± (SD) weight of rats used in the *in vivo* pharmacokinetic studies

Study	n (group)	n (total)	Weight
1. Pharmacokinetic study			
1.1. Intact rats			
1.1.1. Female rats	10	10	249 (44)
1.1.2. Male rats	10	10	376 (36)
1.2. Bile-duct cannulated rats			
1.2.1. Female rats	3	3	310 (79)
1.2.2. Male rats	3	3	334 (92)
2. Gonadectomy and sex hormone study[1]			
2.1. Female rats	4	16	253 (26)
2.2. Male rats	4	16	329 (28)
3. Intestinal permeability study			
3.1. Female rats	6	6	244 (23)
3.2. Male rats	6	6	303 (25)
4. Probenecid and ketoprofen pharmacokinetic study			
4.1. Female rats	3 (6 control)	24	264 (24)
4.2. Male rats	4	8	292 (31)
5. Probenecid and ketoprofen glucuronide pharmacokinetic study			
5.1. Intact female rats	4	8	296 (19)
5.2. Bile-duct cannulated female rats	3	6	307 (24)

[1] Data is for Week 1; see also **Figure 16**

during each dosing experiment. In all rats, urine was collected from 0-24 h following the ketoprofen dose into containers previously rinsed with 1 M HCl to prevent spontaneous cleavage of the glucuronide conjugates. All specimens were frozen at - 20 °C until analysis.

2.3.2.2.2. Sex hormones

The rats were dosed with either estradiol valerate (1 mg/kg) or testosterone cypionate (10 mg/kg) *via* the intramuscular route once a week on the same day for the duration of the experiment.

2.3.2.3. Intestinal permeability study

Rac-ketoprofen (3 mg/kg) in 2% methylcellulose was administered *via* the oral route to male and female rats (n = 6 rats each) whereupon rats were subsequently housed in individual metabolic cages. Three h after the ketoprofen dose, 0.5 mL of an oral solution containing 10 μCi/mL of ^{51}Cr complexed with EDTA was administered to each rat to investigate small intestinal permeability changes. Urine was collected from 0-4, 4-8 and 8-24 h following the ^{51}Cr-EDTA dose into collection vials containing 1 mL of 1 M H_2SO_4 to inhibit microbial growth. After each collection period, cages were washed with 10 mL of tap water and the total urine sample and wash transferred to scintillation vials. Samples were counted utilizing a Beckman Gamma 8000 (Irvine, CA) gamma counter for 1 min in a counting window

scanning within a range of 0-2 MeV. Two standards of 100 μL of the administered ^{51}Cr-EDTA solution were counted with each set of urine samples. Prior to the experiment, baseline permeability was determined on the day prior to dosing with ketoprofen by administering 0.5 mL of a 10 μCi/mL oral solution of ^{51}Cr-EDTA to each rat and similarly collecting and quantitating activity in urine. The relative permeability change from baseline was quantitated by calculating the radioactivity present in each sample as a percentage of the administered ^{51}Cr-EDTA dose after correction for background radiation. Apparent permeability is reported as percent ^{51}Cr-EDTA dose excreted in urine from 0-8 h.

2.3.2.4. Probenecid and ketoprofen pharmacokinetics

2.3.2.4.1. Female rats

Probenecid was prepared by a previously reported method involving dissolution in 1 M NaOH, dilution with physiological saline and readjustment of pH to 7.4 with 0.1 M HCl (Emanuelson and Paalzow, 1986). In female rats, probenecid doses of 0 (saline), 25, 50, 100, 150, 175, and 200 mg/kg (n = 3 rats each dose) were administered as *i.v.* bolus doses 30 min prior to an *i.v.* bolus dose of rac-ketoprofen (10 mg/kg) dissolved in PEG 400. Blood (0.2 mL) was collected from the jugular vein cannula at 0 (30 min after probenecid dose), 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 9.0, 12.0 and 24.0 h after administration of ketoprofen. After dosing and between each

blood sample collection the catheter was flushed with heparin (100 U/mL). The blood was centrifuged and the plasma portion separated. Urine was collected from 0-24 h after the ketoprofen dose into containers previously rinsed with 1M HCl. All specimens were immediately frozen at -20 °C until analysis.

2.3.2.4.2. Male rats

In male rats, probenecid doses of (0) saline or 100 mg/kg probenecid (4 rats each dose) were administered as *i.v.* bolus doses 30 min prior to an *i.v.* bolus dose of rac-ketoprofen (10 mg/kg). Blood (0.2 mL) was collected from the jugular vein cannula and the plasma portion separated and stored as per the previous experiment in female rats detailed in *2.3.2.4.1*.

2.3.2.5. Probenecid and ketoprofen glucuronide pharmacokinetics

2.3.2.5.1. Intact rats

A probenecid dose of 0 (saline) or 100 mg/kg was administered as an *i.v.* dose to jugular vein cannulated female rats (n = 4 each) 30 min before administration of an *i.v.* bolus dose of a mixture of ≈ 1.0 mg/kg each of R-ketoprofen glucuronide and S-ketoprofen glucuronide obtained from urine of a healthy human male volunteer following a 200 mg dose of Orudis E™ (Rhone Poulenc Pharma Inc., Montreal, QC). Prior to injection, the urine was filtered through a 0.45 μm filter attached to the syringe (Fisher Scientific,

Edmonton, AB). The concentrations of the filtered enantiomer glucuronides and any deconjugated enantiomer arising from spontaneous hydrolysis were determined by HPLC analysis immediately prior to dosing and a volume adjustment made to ensure all rats received an equivalent mg/kg dose of the glucuronides. Blood (0.2 mL) was collected from the jugular vein cannula at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 9.0, 12.0 and 24.0 h after administration of the glucuronides. After dosing and between each blood sample collection, the catheter was flushed with heparin (100 U/mL). The blood was centrifuged and the plasma portion separated. Urine was collected from 0-24 h after administration of the enantiomer glucuronides into containers previously rinsed with 1 M HCl. All specimens were immediately frozen at -20 °C in acid rinsed containers and kept frozen until analyzed to prevent spontaneous cleavage of the glucuronides.

2.3.2.5.2. Bile-duct cannulated rats

A similar protocol as in *2.3.2.5.1.* was followed where *i.v.* bolus doses of either 0 (saline) or 100 mg/kg probenecid were given 30 min prior to an *i.v.* dose of a mixture of R-ketoprofen glucuronide and S-ketoprofen glucuronide to jugular vein and bile-duct cannulated female rats (n = 3 rats each dose). Blood (0.2 mL) was collected at 0.25, 0.5, 1.0, 1.5, 2.0 and 3.0 h and bile collected from 0-3 h into 1 M HCl rinsed containers. All specimens were immediately frozen at -20 °C and kept frozen until analysis.

Attempts to collect urine from 0-3 h were unsuccessful due to negligible urine output in these rats.

2.4 *In vitro* studies

2.4.1. Protein binding

2.4.1.1. Apparatus and techniques

An Amicon Micropartition Ultrafiltration system (MPS-1, Amicon, Beverly, MA) with YMT membranes (No. 40420) was used to evaluate the *in vitro* binding of ketoprofen enantiomers to male and female rat plasma. Triplicate samples of each concentration: 25, 50, 100 and 200 μg/mL of rac-ketoprofen in plasma were incubated for 4 h at 37 °C. The total volume of each sample was 1 mL. A 100 μL aliquot of each sample was removed prior to ultrafiltration in order to determine total drug concentration and 500 μL of the sample was pipetted into the ultrafiltration system. The ultrafiltration system was capped and centrifuged for 20 minutes in a Clay-Adams centrifuge set at 1800 x ġ force thereby obtaining ≈ 400 μL of ultrafiltrate. A 300 μL aliquot of the ultrafiltrate was removed for analysis of the unbound fraction of R- or S-ketoprofen. Preliminary studies indicated that ketoprofen did not bind to the ultrafiltration device.

2.4.2. Glucuronidation

2.4.2.1. Microsomal preparation

Hepatic and renal microsomes were prepared from the rats used in the gonadectomy and sex hormone study after completion of the experiment (*see 2.3.2.2.*). Microsomes were prepared from control, ovarectomized, ovarectomized + testosterone and ovarectomized + estradiol treated female rats (n = 4 rats each) and from control, castrated, castrated + estradiol and castrated + testosterone treated male rats (n = 4 rats each). Rats were anesthetized with diethyl ether (Caledon Laboratories, Georgetown, ON) and all 4 lobes of the liver and both kidneys were excised from each animal *via* midline incision and immediately placed in ice-cold 1.15% KCl solution. Individual tissues from the 4 rats in each group were pooled and homogenized with 2 volumes of ice-cold 100 mM phosphate buffer (pH 7.4) containing 250 mM sucrose, all the while keeping the tissue samples on ice. The homogenized tissue was centrifuged at 5 °C for 20 min at 10,000 x ġ in a Beckman model L855 centrifuge (Beckman, Palo Alto, CA). The supernatant was then centrifuged at 5 °C for 60 min at 105,000 x ġ. The supernatant was discarded and the solid pellet resuspended in 2 volumes of 100 mM phosphate:250 mM sucrose buffer and again centrifuged at 5 °C for 60 min at 105,000 x ġ. The supernatant was again discarded and the resulting pellet resuspended in 2 volumes of phosphate:sucrose buffer and frozen at -20 °C until use. The liver and kidney microsomal protein

concentration of each group of microsomes was determined in triplicate by the method of Lowry *et al.*, 1951 (Protein Assay Kit No. P5656, Sigma Diagnostics, St. Louis, MO).

2.4.2.2. Microsomal incubation

2.4.2.2.1. Ketoprofen

Glucuronidation of 50 μg/mL (200 μM) rac-ketoprofen was determined in triplicate in each group of microsomes as this concentration is in accordance to what is seen in plasma following 10 mg/kg rac-ketoprofen given *i.v.* to rats. For each incubation, 2.0 mg liver or kidney microsomal protein was incubated with reaction mixtures containing 20 mM UDPGA and 5mM $MgCl_2$ and 0.05% Triton-X in 100 mM phosphate and 250 mM sucrose buffer (pH 7.4) for 10 min at 37 °C in a shaking water bath. Preliminary work indicated that glucuronidation was linear under these conditions with respect to time, ketoprofen, UDPGA and protein concentrations. Reactions were started by addition of rac-ketoprofen (50 μg/mL in reaction mixture) and run for 60 min. The total incubation volume was 1.0 mL. Samples (100 μL) were taken at 5, 15, 30 and 60 min and transferred to clean test tubes containing either 100 μL 1 M NaOH (hydrolyzed) or 150 μL 0.6 M H_2SO_4 (unhydrolyzed). To the basified samples, 200 μL of 0.6 M H_2SO_4 was added to enable extraction and then all samples were immediately analyzed. The difference between basified/acidified (hydrolyzed) and acidified (unhydrolyzed) samples

was taken to be the amount of ketoprofen enantiomer metabolized to glucuronide conjugate.

2.4.2.2.2. Ketoprofen and probenecid

Glucuronidation of 200 μM (50 μg/mL) rac-ketoprofen was determined in triplicate in the absence and presence of either 40 μM ($\approx$ 10 μg/mL), 200 μM ($\approx$ 50 μg/mL) or 1000 μM ($\approx$ 250 μg/mL) concentrations of probenecid. For each incubation, 2.0 mg liver or kidney control female microsomal protein was incubated with reaction mixtures containing 20 mM UDPGA and 5mM $MgCl_2$ and 0.05% Triton-X in 100 mM phosphate and 250 mM sucrose buffer (pH 7.4) for 10 min at 37 °C in a shaking water bath. Reactions were started by addition of rac-ketoprofen and probenecid and run for 60 min. The total incubation volume was 1.0 mL. Samples (100 μL) were taken at 5, 15, 30 and 60 min and transferred to clean test tubes containing 100 μL 1M NaOH (hydrolyzed) or 150 μL 0.6M H_2SO_4 (unhydrolyzed). To the basified samples, 200 μL of 0.6M H_2SO_4 was added to facilitate extraction and then all samples were immediately analyzed. The difference between basified/acidified (hydrolyzed) and acidified (unhydrolyzed) samples was taken to be the amount of ketoprofen enantiomer glucuronide conjugate.

2.5. Data analysis

2.5.1. Pharmacokinetic indices

The area under the plasma concentration time curve (AUC) was calculated using the linear trapezoidal rule from 0 to t hours where t represents the time of the last concentration point (C_{last}). For determination of $AUC_{(0-\infty)}$ the area from C_{last} to infinity was calculated as C_{last}/β where β is the terminal elimination rate constant determined by the best fit line through the log linear terminal portion of the curves. Elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/\beta$ and total body clearance (CL_{TB}) was determined as CL_{TB} = Dose/AUC. Volume of distribution (V_d) was calculated as $V_d = CL_{TB}/\beta$ (Gibaldi and Perrier, 1982).

The percentage of dose as glucuronide conjugate recovered in urine or bile was calculated as amount of R- or S-ketoprofen glucuronide recovered in the specimen divided by the enantiomer dose and multiplied by 100. The fraction of AUC attributed to enterohepatic recirculation was calculated as Mean AUC_{Intact} - Mean AUC_{BC} / Mean AUC_{Intact} where AUCs are mean values from 0 to 9 hours. Clearance of ketoprofen through glucuronidation and subsequent biliary excretion (CL_{bconj}) was calculated as the amount of R- or S-ketoprofen glucuronide recovered in bile over 9 h divided by the corresponding $AUC_{(0-9h)}$ of unchanged enantiomer in plasma assuming rapid biliary excretion following formation of the glucuronide. Similarly, clearance through glucuronidation and subsequent urinary excretion (CL_{uconj}) was

calculated as the amount of R- or S-ketoprofen glucuronide recovered in urine over 24 h divided by the corresponding $AUC_{(0\text{-}24h)}$ of unchanged enantiomer in plasma assuming rapid urinary excretion following appearance of glucuronide in plasma.

In the probenecid concentration dependency study, the AUC of probenecid was calculated using the linear trapezoidal rule from 0.5-24.5 h due to the limitation of the number of samples that could be safely taken without compromising the animals. To avoid error in extrapolating back to determine the concentration at time zero, we chose to consider the probenecid AUC only during the presence of ketoprofen. Mean residence time (MRT) was calculated as AUMC/AUC where AUMC is the area under the curve of a plot of the product of concentration of probenecid and time *vs* time from 0.5 h to 24.5 h.

The amount of weight gained in gonadectomized and sex hormone treated rats was calculated as the difference in body weight from week 1 multiplied by 100%. In the microsomal studies, the amount of ketoprofen enantiomer glucuronide formed is reported as the μmol/mg protein formed in 60 min (1 h).

2.5.2. Statistical analysis

Significant differences in the pharmacokinetic parameters of R- and S-ketoprofen between female and male rats and between intact and bile-duct

cannulated rats were investigated by the independent measures Student's t test. Significant differences in urinary recovery of the dose of ^{51}Cr-EDTA between female and male rats were also investigated by the independent measures Student's t test and differences between baseline and post-ketoprofen dose recovery of ^{51}Cr-EDTA in the same rats were investigated by the repeated measures Student's t test.

In the probenecid - ketoprofen interaction study, significance of the correlation coefficents (r) obtained were determined by comparison of the value obtained from the best fit regression line with published tables of significant values (Steele and Torrie, 1960). Differences in pharmacokinetic parameters of R- and S-ketoprofen resulting from the various doses of probenecid were examined using the one way ANOVA. One rat (175 mg/kg probenecid dose) was excluded from calculations requiring plasma concentrations after 4.0 h as the jugular vein cannula ceased to work after this time.

In the gonadectomy and sex hormone treatment study, significant differences in the urinary excretion of S-ketoprofen glucuronide between treatment groups and within treatment groups with time were examined by use of the two-way, repeated-measures ANOVA in male and female rats, respectively.

For the protein binding study, significant differences in the unbound fraction of ketoprofen enantiomer between male and female rats and

between different concentrations were examined by use of the two-way ANOVA. For the microsomal studies, significant differences between groups in the amount of glucuronide formed were examined by use of the one-way ANOVA.

In studies where significant differences were found utilizing one-way or two-way ANOVA, the Duncan's multiple range test was used to compare means (Knudsen-Randolph and Ciminera, 1980). All tests were conducted at the $\alpha = 0.05$ level of significance. In all cases, unless otherwise noted, results are expressed as mean $\pm$ (SD).

3. RESULTS

3.1. Assays

3.1.1. Ketoprofen assay

Retention times for the R- and S-indoprofen diastereomers were 6.5 and 7.9 min, respectively. Overall, the modifications to the assay of Foster and Jamali, 1987 resulted in reduced run time and improved resolution of the diastereomer peaks due to the changes in mobile phase.

3.1.2. Ketoprofen and probenecid assay

In Figure 3 is depicted chromatograms of blank rat plasma, plasma spiked with 10 μg/mL of each ketoprofen enantiomer and probenecid and a 6 hour rat plasma sample. The diastereomers of R- and S-indoprofen and R- and S-ketoprofen and probenecid elute at 6.5, 7.9, 10.7, 12.6 and 19.4 min, respectively. The orders of elution of the enantiomers of indoprofen (Bjorkman, 1985) and ketoprofen (Jamali and Foster, 1987) have previously been determined. The 6 hour plasma sample had 0.65 μg/mL R-ketoprofen, 16.07 μg/mL S-ketoprofen and 1.39 μg/mL probenecid. The chromatograms represented in Figure 4 are of blank rat urine, urine spiked with 5 μg/mL of each ketoprofen enantiomer and probenecid and unhydrolyzed and hydrolyzed urine samples collected from 6-9 hours after ketoprofen dosing. The unhydrolyzed sample had 0.99 μg/mL R-ketoprofen, 2.17 μg/mL S-ketoprofen and 55.27 μg/mL probenecid and when hydrolyzed an identical

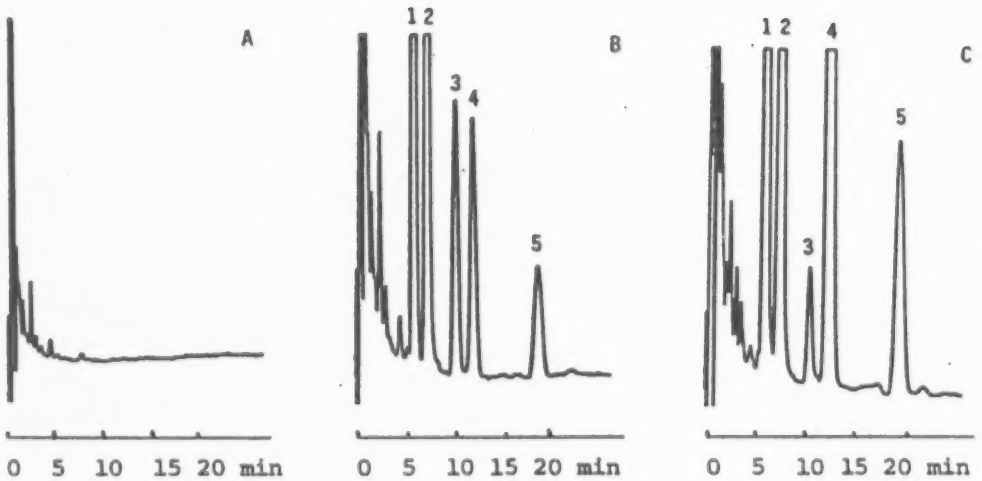

Figure 3. HPLC profiles of blank plasma (A); plasma spiked with 10 μg/mL of each enantiomer of ketoprofen and probenecid (B) and a 6h plasma sample after administration of an *i.v.* dose of 50 mg/kg probenecid and 10 mg/kg rac-ketoprofen in a female rat (C). Peaks: 1, R-internal standard; 2, S-internal standard; 3, R-ketoprofen; 4, S-ketoprofen; 5, probenecid.

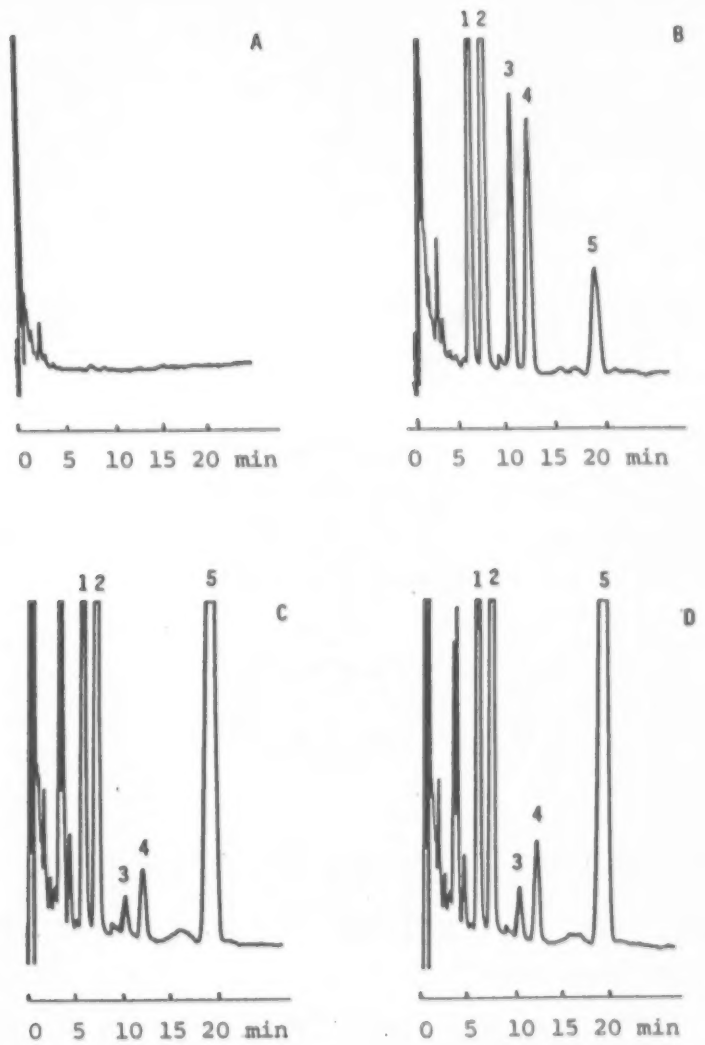

Figure 4. HPLC profiles of blank urine (A), urine spiked with 5 μg/mL of each enantiomer of ketoprofen and probenecid (B); a 6-9 h unhydrolyzed urine sample (C) and a 6-9 h hydrolyzed urine sample (D) after administration of an *i.v.* dose of 50 mg/kg probenecid and 10 mg/kg rac-ketoprofen in a female rat. Peaks: 1, R-internal standard; 2, S-internal standard; 3, R-ketoprofen; 4, S-ketoprofen; 5, probenecid.

sample yielded 1.19 μg/mL R-ketoprofen, 2.91 μg/mL S-ketoprofen and 59.26 μg/mL probenecid.

There are no interfering peaks. The first eluting peak of internal standard (Peak 1; Figures 3 and 4) was used for quantitation. Typical best fit regression equations for lines passing through the plasma and urine data points are given in Table IV.

The assay is accurate and reproducible as the coefficients of variation (CV) obtained for intraday and interday variability over the examined concentration range (1-40 μg/mL) of plasma and urine were less than 10% in each instance.

The detection limit of R-ketoprofen, S-ketoprofen and probenecid was below 0.5 μg/mL; however, reproducible results were obtained only when the concentration was 0.5 μg/mL or higher. Thus, the minimum quantifiable concentration (MQC) using this assay is 0.5 μg/mL ($n = 4$, $CV < 9.54\%$) which was found to be sufficient for determination of concentrations in 100 μL rat plasma samples 12-24 h post dosing.

3.1.2.1. Extraction yields

Retention times were 3.4, 4.7 and 6.5 min respectively for underivatized indoprofen, ketoprofen and probenecid. The extraction yields were 85.81 $\pm$ 2.8% and 86.86 $\pm$ 7.4% from plasma and 92.78 $\pm$ 6.2% and 83.88 $\pm$ 7.2% from urine for ketoprofen and probenecid respectively.

Table IV. Typical regression equations for R-ketoprofen, S-ketoprofen and probenecid (1-40 μg/mL) together and alone in plasma and urine samples

Regression Equations[1]				
Compound	Together	r^2	Alone	r^2
A) Plasma (n = 9)				
R-ketoprofen	y = 0.0151x + 0.0025	.999	y = 0.0163x - 0.0069	.999
S-ketoprofen	y = 0.0154x + 0.0033	.999	y = 0.0169x - 0.0059	.999
Probenecid	y = 0.0093x + 0.0023	.999	y = 0.0089x - 0.0007	.999
B) Urine (n = 3)				
R-ketoprofen	y = 0.0140x + 0.0002	.999	y = 0.0259x - 0.0088	.999
S-ketoprofen	y = 0.0143x + 0.0001	.999	y = 0.0266x - 0.0087	.999
Probenecid	y = 0.0083x + 0.0080	.998	y = 0.0125x + 0.0148	.997

[1] x = drug concentration; y = peak area ratio of drug/internal standard

3.1.2.2. Derivatization yields

Derivatization yields obtained were 99.15 $\pm$ 0.3%, 97.54 $\pm$ 0.7% and 84.04 $\pm$ 6.2% for indoprofen, ketoprofen and probenecid respectively.

3.1.2.3. Standard curves and accuracy

The accuracy of the analytical method was evaluated by comparison of theoretical and calculated concentrations; the results are given in Table V.

3.1.2.4. Other compounds tested

Numerous compounds were examined for their suitability as an internal standard as detailed in *2.2.3.2.4.* Although all were derivatized by this method and satisfactory resolution of individual diastereomers noted for fenoprofen, cicloprofen and pirprofen, only flurbiprofen, indoprofen and carprofen diastereomers did not interfere with any of the ketoprofen diastereomer or probenecid peaks. This method was found suitable for the simultaneous determination of indoprofen, carprofen and flurbiprofen enantiomers with probenecid in biological samples using ketoprofen as an internal standard. To quantify flurbiprofen enantiomers; however, only the R-ketoprofen peak could be used because of the interference of an impurity peak of flurbiprofen with S-ketoprofen.

Table V. Evaluation of accuracy for ketoprofen and probenecid assay

Compound	Theoretical concentration (μg/mL)	Mean calculated concentration (μg/mL) (n = 9)	Coefficient of variation (%)
R-ketoprofen	1.0	0.970	9.144
S-ketoprofen	1.0	0.967	8.319
Probenecid	1.0	1.031	8.619
R-ketoprofen	10.0	10.477	5.793
S-ketoprofen	10.0	10.451	6.207
Probenecid	10.0	10.470	2.729
R-ketoprofen	40.0	40.042	0.376
S-ketoprofen	40.0	40.091	0.412
Probenecid	40.0	40.046	0.748

3.2. Gender dimorphism in pharmacokinetics of ketoprofen in rats

3.2.1. Pharmacokinetic studies in female and male rats

3.2.1.1. Intact rats

Marked stereoselectivity in the disposition of ketoprofen enantiomers was found in both sexes following 10 mg/kg *i.v.* bolus doses of rac-ketoprofen (Figure 5). Significantly larger AUC and lower CL_{TB} values were found in female than in male rats for both ketoprofen enantiomers (Table VI). Consistent with previous findings in the male rat, plasma levels of R- or S-ketoprofen glucuronides in either sex were negligible (Foster and Jamali, 1987).

There were no significant gender differences in S:R ketoprofen AUC ratio or $t_{1/2}$ of either ketoprofen enantiomer (Table VI). The V_d of R-ketoprofen was significantly smaller in the female when compared with the male. Similarly, a trend towards smaller S-ketoprofen V_d in the female was observed but failed to reach statistical significance (Table VI).

The mean cumulative recovery in urine (ΣXu_{urine}) of R-ketoprofen glucuronide was 3.23 $\pm$ 1.53% and 2.34 $\pm$ 3.22% of the enantiomer dose from female and male rats, respectively (Figure 6). The mean ΣXu_{urine} of S-ketoprofen glucuronide from male rats was 8.20 $\pm$ 5.81%, similar to previous results (Table II), however, the amount recovered in female rat urine was approximately 4 times greater (39.61 $\pm$ 18.20 %) (Figure 6).

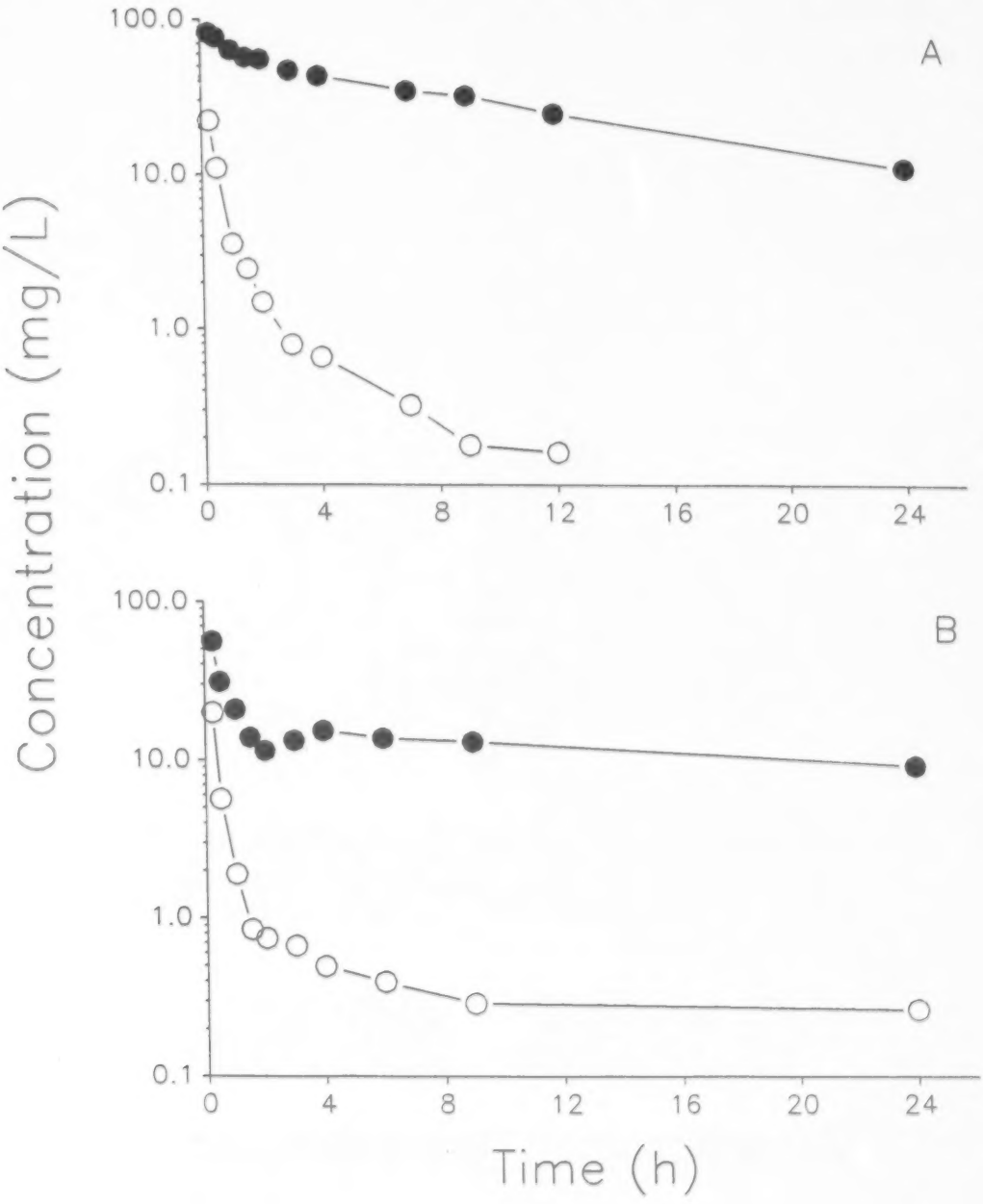

Figure 5. Plasma concentration *vs* time profiles of R-ketoprofen (○) and S-ketoprofen (●) after administration of 10 mg/kg rac-ketoprofen *i.v.* in a representative intact female (A) and male (B) rat.

Table VI. Gender differences in mean ± (SD) pharmacokinetic indices of ketoprofen enantiomers in the rat

A) Intact Rats:

	$AUC_{(0-\infty)}$ (mg.h/L)			$t_{1/2}$ (h)		V_d (L)		CL_{TB} (mL/min)	
	R	S	S:R	R	S	R	S	R	S
Female (n = 9):									
Mean	25[a]	466[ab]	19[a]	15.2[a]	17.5[a]	1.19[ab]	0.08	0.89[a]	0.05[ab]
SD	(7.1)	(196)	(6.2)	(4.5)	(8.9)	(0.49)	(0.06)	(0.18)	(0.02)
Male (n = 9):									
Mean	16[ab]	284[ab]	22.2[a]	18.1[a]	16.0[a]	3.08[ab]	0.15	2.05[a]	0.11[ab]
SD	(3.3)	(43)	(10.1)	(9.2)	(8.1)	(1.49)	(0.07)	(0.32)	(0.02)

B) Bile-Duct Cannulated Rats:

	$AUC_{(0-\infty)}$ (mg.h/L)			$t_{1/2}$ (h)		V_d (L)		CL_{TB} (mL/min)	
	R	S	S:R	R	S	R	S	R	S
Female (n = 3):									
Mean	54[a]	185[ab]	3.3[a]	2.7[ab]	5.8[ab]	0.11[a]	0.07	0.59[a]	0.20[ab]
SD	(21)	(102)	(0.6)	(1.2)	(2.7)	(0.04)	(0.02)	(0.25)	(0.12)
Male (n = 3):									
Mean	10[ab]	44[ab]	4.6[a]	0.5[ab]	0.9[ab]	0.09[a]	0.05	2.60[a]	0.58[ab]
SD	(2.8)	(4.4)	(0.9)	(0.3)	(0.7)	(0.05)	(0.04)	(0.38)	(0.10)

[a]significant difference (female *vs* male); [b]significant difference (intact *vs* bile-duct cannulated)
$\alpha = 0.05$

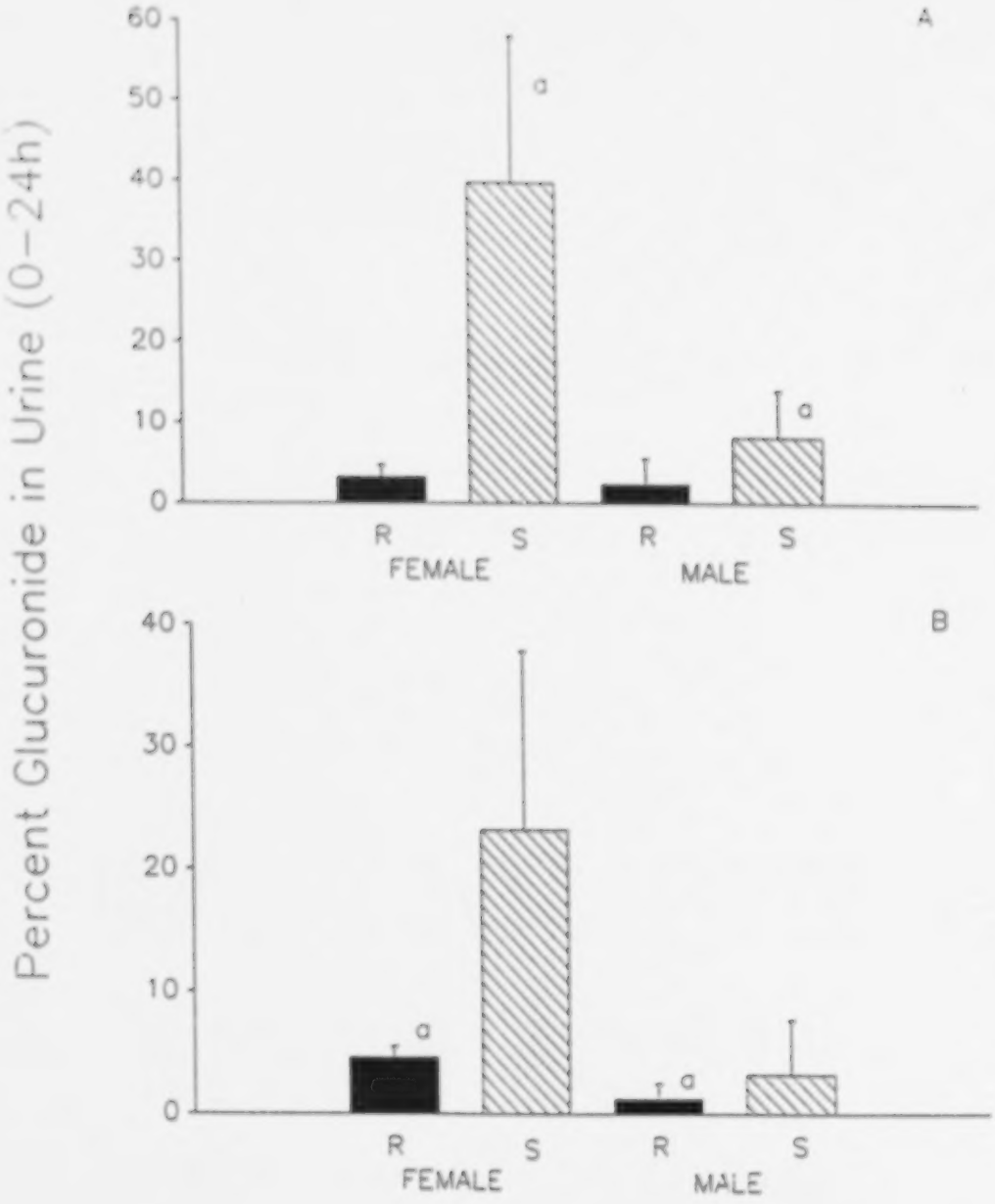

Figure 6. Cumulative urinary excretion of R-ketoprofen glucuronide (■) and S-ketoprofen glucuronide (▨) in intact (n = 9) (A) and bile-duct cannulated (n = 3) (B) female and male rats. Data are presented as the mean ± SD. The letter (a) represents a significant difference (female *vs* male) $\alpha = 0.05$.

The S:R ketoprofen glucuronide ratio in urine of females (13.7 ± 7.8) was more than double that observed in males (6.4 ± 4.2). Urinary recovery of non-glucuronidated R- or S-ketoprofen was negligible.

3.2.1.2. Bile-duct cannulated rats

The disposition of ketoprofen enantiomers in both sexes showed marked stereoselectivity following bile-duct cannulation (Figure 7). In male rats, the AUC of both ketoprofen enantiomers was significantly lower than in intact male rats indicating the large contribution of enterohepatic recirculation to enantiomer AUC (Table VI). In bile-duct cannulated female rats, S-ketoprofen AUC was also significantly lower than in intact female rats. Interestingly, the mean S-ketoprofen AUC following bile-duct cannulation when compared with AUC in intact animals was reduced only 1.5 fold in female rats as opposed to a 5.5 fold reduction in male rats (Table VI). The mean AUC of R-ketoprofen in bile-duct cannulated females, although higher than mean AUC in intact females, was not significantly different (Table VI).

S:R ketoprofen AUC ratios did not differ between sexes, although S:R ratios were significantly lower in bile-duct cannulated than intact animals (Table VI). The $t_{1/2}$ values of both ketoprofen enantiomers were significantly shorter in bile-duct cannulated rats when compared to intact rats, regardless of sex (Table VI). The $t_{1/2}$ values of both enantiomers were significantly shorter in bile-duct cannulated males when compared with females.

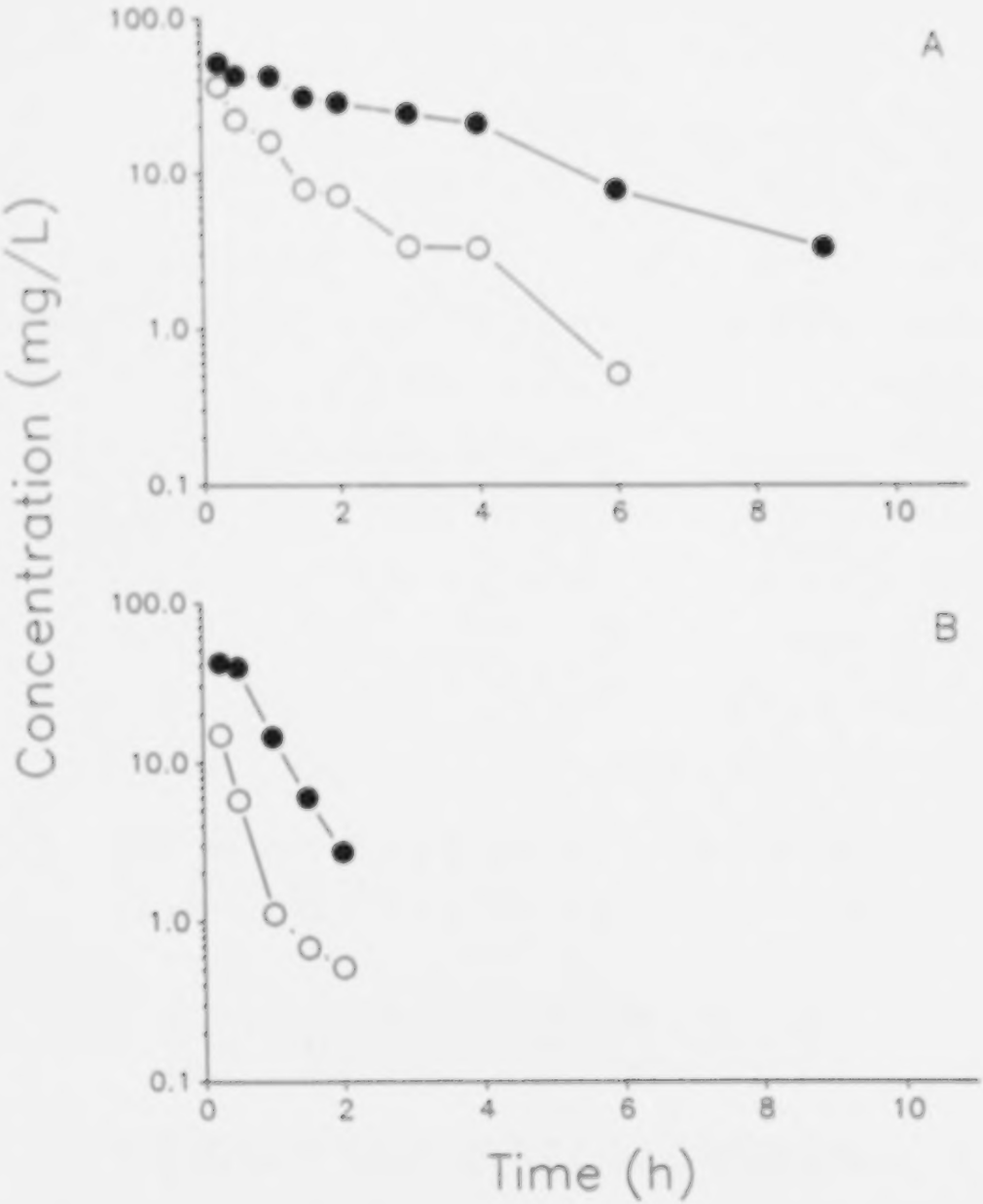

Figure 7. Plasma concentration *vs* time profiles of R-ketoprofen (○) and S-ketoprofen (●) after administration of 10 mg/kg rac-ketoprofen *i.v.* in a representative bile-duct cannulated female (A) and male (B) rat.

No significant gender difference in R- or S-ketoprofen V_d was found in bile-duct cannulated rats (Table VI). In both sexes, a significantly smaller R-ketoprofen V_d was found after bile-duct cannulation when compared with intact rats. S-ketoprofen V_d did not appear to change in bile-duct cannulated females, however, in male rats a trend towards smaller S-ketoprofen V_d when compared with intact males was observed although the difference in values was not significant (Table VI).

The ΣXu_{urine} of R-ketoprofen glucuronide was 4.59 $\pm$ 0.94% and 1.19 $\pm$ 1.31% of the enantiomer dose, respectively, from female and male rats (Figure 6). The ΣXu_{urine} of S-ketoprofen glucuronide in females (23.19 $\pm$ 14.63%) was higher than in males (3.23 $\pm$ 4.52%), although the values were not statistically significant (Figure 6). The urinary recovery of non-glucuronidated enantiomers was negligible.

There were no significant gender differences in the overall mean cumulative recovery in bile (ΣXu_{bile}) of R- or S-ketoprofen glucuronide (Figure 8). The S:R ratio of the enantiomer glucuronides in bile in males (12.2 $\pm$ 2.4) was found to be almost double that in females (6.8 $\pm$ 1.3). In addition, the mean clearance of S-ketoprofen glucuronide via bile (CL_{bile}) was found to be significantly slower in female (0.20 $\pm$ 0.14 mL/min) than in male rats (0.67 $\pm$ 0.16 mL/min). The mean CL_{bile} of R-ketoprofen in females (0.10 $\pm$ 0.07 mL/min) although less than males (0.25 $\pm$ 0.05 mL/min), was not statistically different.

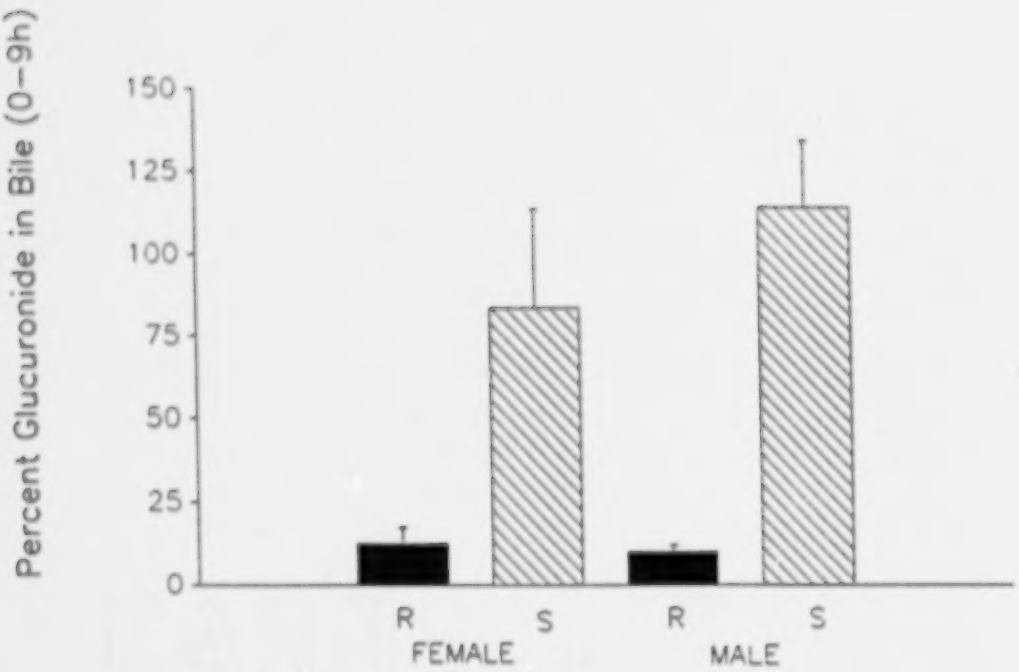

Figure 8. Cumulative biliary excretion of R-ketoprofen glucuronide (■) and S-ketoprofen glucuronide (▨) in bile-duct cannulated female and male rats. Data are presented as the mean ± SD (n = 3 rats each group). No significant differences were found (female *vs* male) $\alpha = 0.05$.

In total, 75.7 ± 14.5% (male) and 67.4 ± 31.0% (female) of the dose of rac-ketoprofen was recovered in bile-duct cannulated rats. Total bile and urine output volumes were comparable between the female and male rats.

The fraction of AUC of S-ketoprofen attributed to enterohepatic recirculation was 70% in males and 30% in females. In male rats, the fraction of R-ketoprofen AUC attributed to enterohepatic recirculation during 9 h was 24%. This value was not calculated in female rats due to the unexpected higher R-ketoprofen AUC in bile-duct cannulated female rats.

The excretion rate of R-ketoprofen glucuronide into bile was found to be significantly slower in the female during all bile collection intervals than in the male (Figure 9) . Significant gender differences in excretion rate of S-ketoprofen glucuronide into bile were not found during the first bile collection period (0-3h), however in subsequent collections, a significantly slower excretion rate was observed in the female (Figure 9). The corresponding mean $t_{1/2}$s of excretion into bile for male and female rats, respectively, were 0.91 h and 1.45 h for R-ketoprofen glucuronide and 1.39 h and 6.20 h for S-ketoprofen glucuronide. R-ketoprofen glucuronide levels were no longer detectable (Figure 9) in male bile after the 6 h collection time.

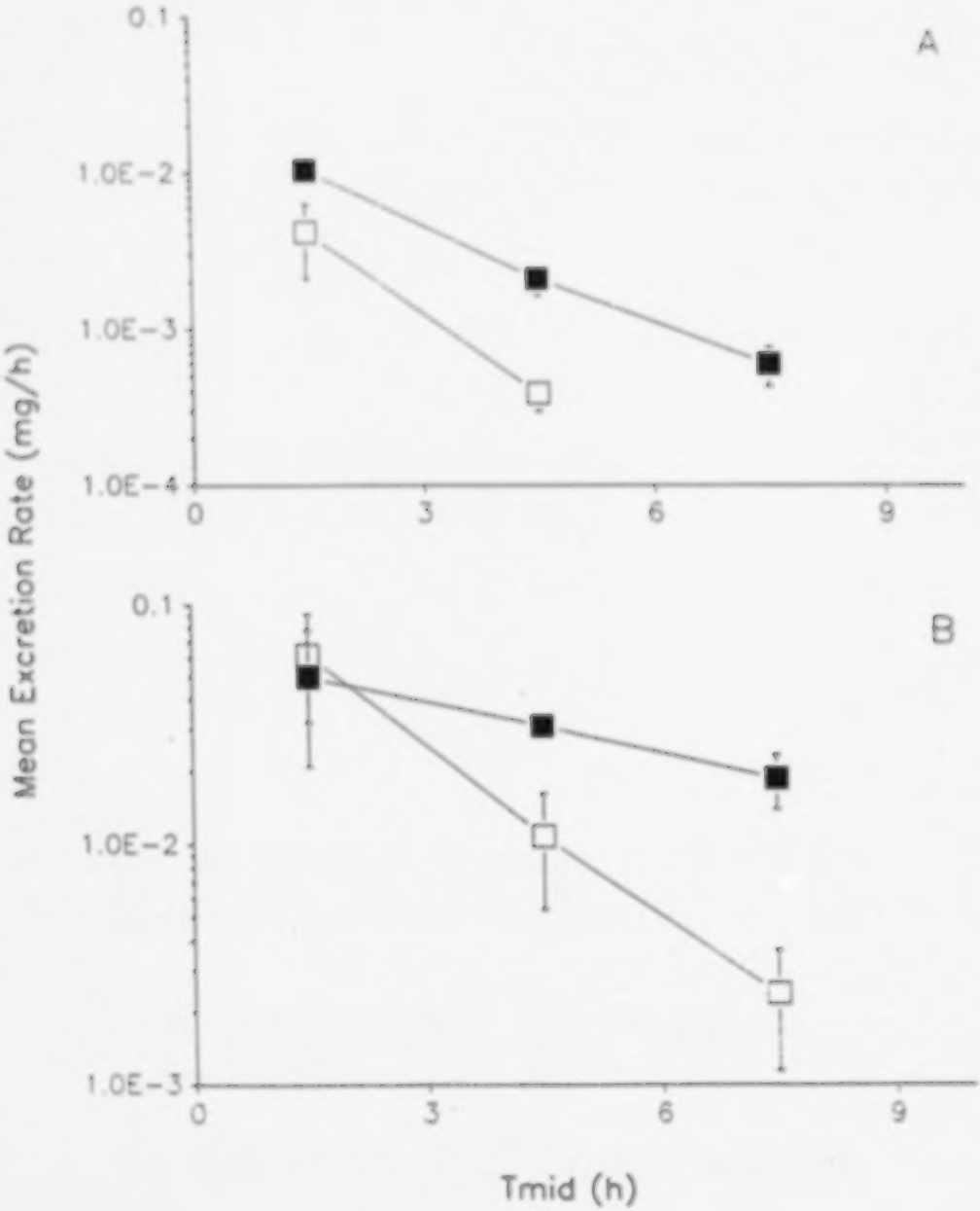

Figure 9. Excretion rate into bile of R-ketoprofen glucuronide (A) and S-ketoprofen glucuronide (B) in bile-duct cannulated female (■) and male (□) rats. Data are presented as the mean ± SD (n = 3 rats).

3.2.2. *In vitro* glucuronidation of ketoprofen

3.2.2.1. Liver microsomal activity

Hepatic microsomes prepared from control male rats had significantly higher UDPGT activity towards both ketoprofen enantiomers than those from control female rats (R: 21.3 ± 5.4; S: 20.5 ± 5.4 *vs* R: 16.6 ± 2.5; S: 14.3 ± 2.7 μmol/h/mg protein), respectively (Figure 10). Overall, hepatic glucuronidation of R- and S-ketoprofen was lowest in microsomes prepared from ovarectomized females (Figure 10) although this was statistically significant only for S-ketoprofen (Table VII). There was significantly higher activity in microsomes of ovarectomized + testosterone treated females than those of either control or ovarectomized rats (Table VII).

There was significantly lower glucuronidation of both R- and S-ketoprofen in microsomes from castrated males when compared with control males (Figure 10, Table VII) whereas hepatic activity of castrated + estradiol treated males was statistically lower than that of control males only for S-ketoprofen (Table VII). Overall, the highest hepatic activity of any microsomal preparation was found in liver microsomes prepared from castrated + testosterone treated males for both ketoprofen enantiomers (R: 27.1 ± 5.7; S: 25.5 ± 4.9 μmol/h/mg protein) (Table VII). This group was also found to have the lowest urinary excretion of S-ketoprofen glucuronide (3.5 ± 1.0% at 4 weeks) of any group in the gonadectomy and sex hormone study (*see 3.2.3.2.*). A significant negative correlation ($r = -0.859$)

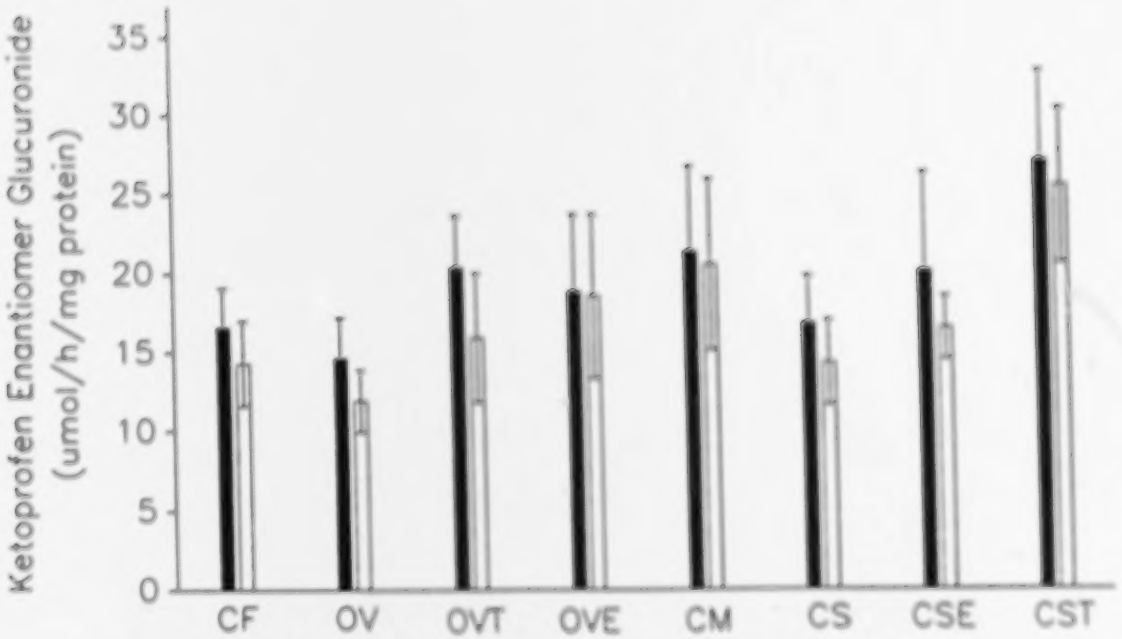

Figure 10. Glucuronidation of R-ketoprofen (■) and S-ketoprofen (□) by liver microsomes derived from control (CF), ovarectomized (OV), ovarectomized + testosterone (OVT) and ovarectomized + estradiol (OVE) treated female rats and control (CM), castrated (CS), castrated + estradiol (CSE) and castrated + testosterone (CST) treated male rats. Data are presented as the mean ± SD (n = 3 incubations). Statistics given in **Table VII**.

Table VII. Duncan's Multiple Range Test[1] results for data in Figures 10 and 12

Figure 10. Liver microsomes

R-ketoprofen glucuronide								S-ketoprofen glucuronide							
OV	CF	CS	OVE	CSE	OVT	CM	CST	OV	CF	CS	OVT	CSE	OVE	CM	CST

Figure 12. Kidney microsomes

R-ketoprofen glucuronide								S-ketoprofen glucuronide							
CST	OV	OVT	OVE	CM	CS	CSE	CF	CST	OVT	OV	OVE	CM	CS	CSE	CF

[1] Ranked lowest to highest; connecting lines indicate insignificant differences $\alpha = 0.05$

CF, control female; OV, ovarectomy; OVE, ovarectomy + estradiol and OVT, ovarectomy + testosterone treated female rats and CM, control male; CS, castrated; CST, castrated + testosterone and CSE, castration + estradiol treated male rats

was found between hepatic microsomal glucuronidation of S-ketoprofen by the gonadectomized and sex hormone treated male and female rats and 24 h urinary recovery of S-ketoprofen glucuronide (Figure 11).

3.2.2.2. Kidney microsomal activity

Significantly higher kidney glucuronidation of both ketoprofen enantiomers was observed in microsomes prepared from control females (R: 6.0 $\pm$ 0.4; S: 5.9 $\pm$ 1.2 μmol/h/mg protein) when compared with those of control males (R: 3.8 $\pm$ 1.9; S: 3.8 $\pm$ 0.6 μmol/h/mg protein) (Figure 12, Table VII). Overall, renal glucuronidation was highest in kidney microsomes prepared from control females when compared with any other preparation (Table VII). Interestingly, control females were also found to have the highest 24 h urinary recovery of S-ketoprofen glucuronide (49.7 $\pm$ 24.1% at 4 weeks) of any group in the gonadectomy and sex hormone treatment study (see *3.2.3.1.*). A trend towards low kidney glucuronidation in testosterone treated rats seemed apparent as the lowest glucuronidation of R- and S-ketoprofen was observed in castrated + testosterone treated males (Table VII). A significant positive correlation ($r = 0.597$) was found between kidney glucuronidation of S-ketoprofen and 24 h urinary recovery of S-ketoprofen glucuronide in the gonadectomized and sex hormone treated rats (Figure 13).

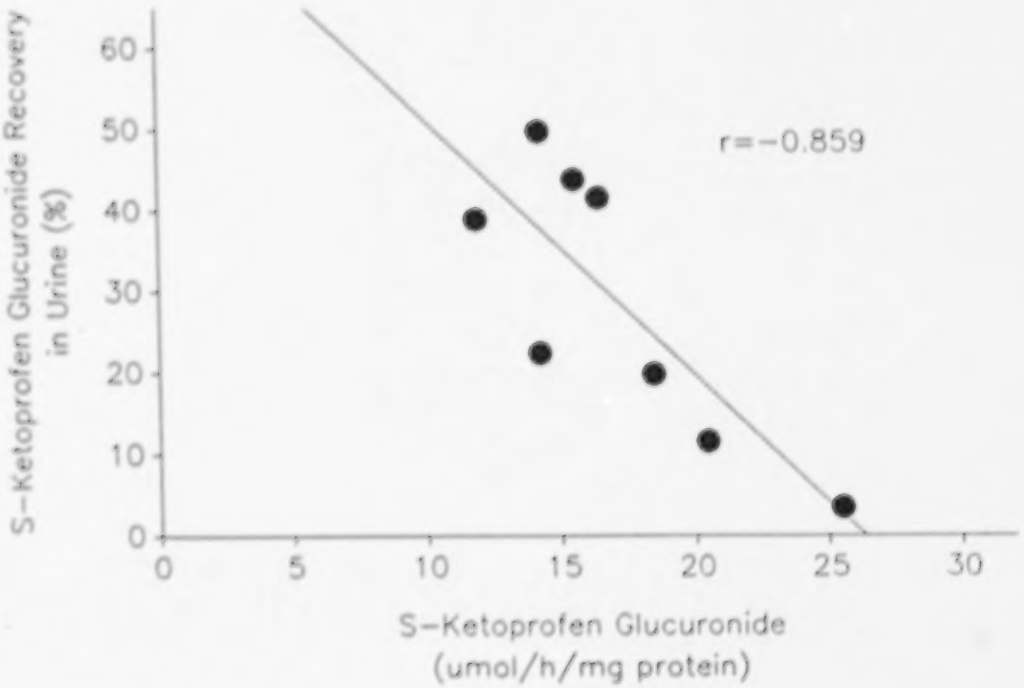

Figure 11. Correlation of mean percent of S-ketoprofen dose as S-ketoprofen glucuronide recovered in 24 h urine collection (n = 4 rats) and mean amount of S-ketoprofen glucuronide formed by liver microsomes (n = 3 incubations) from gonadectomized and sex hormone treated male and female rats.

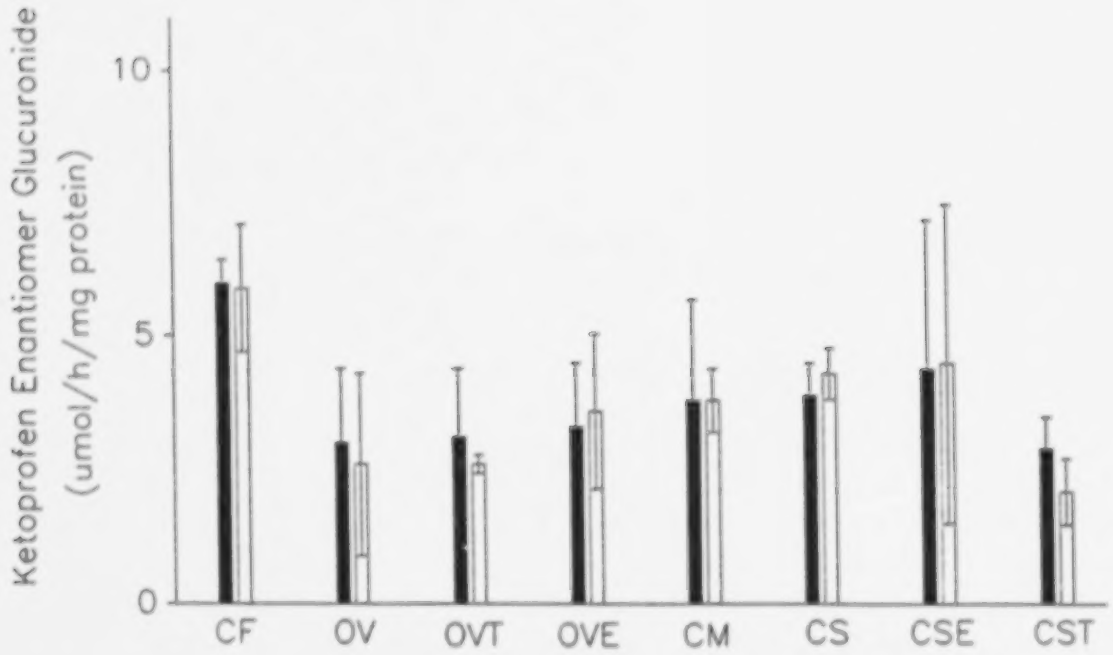

Figure 12. Glucuronidation of R-ketoprofen (■) and S-ketoprofen (□) by kidney microsomes derived from control (CF), ovarectomized (OV), ovarectomized + testosterone (OVT) and ovarectomized + estradiol (OVE) treated female rats and control (CM), castrated (CS), castrated + estradiol (CSE) and castrated + testosterone (CST) treated male rats. Data are presented as the mean ± SD (n = 3 incubations). Statistics given in **Table VII**.

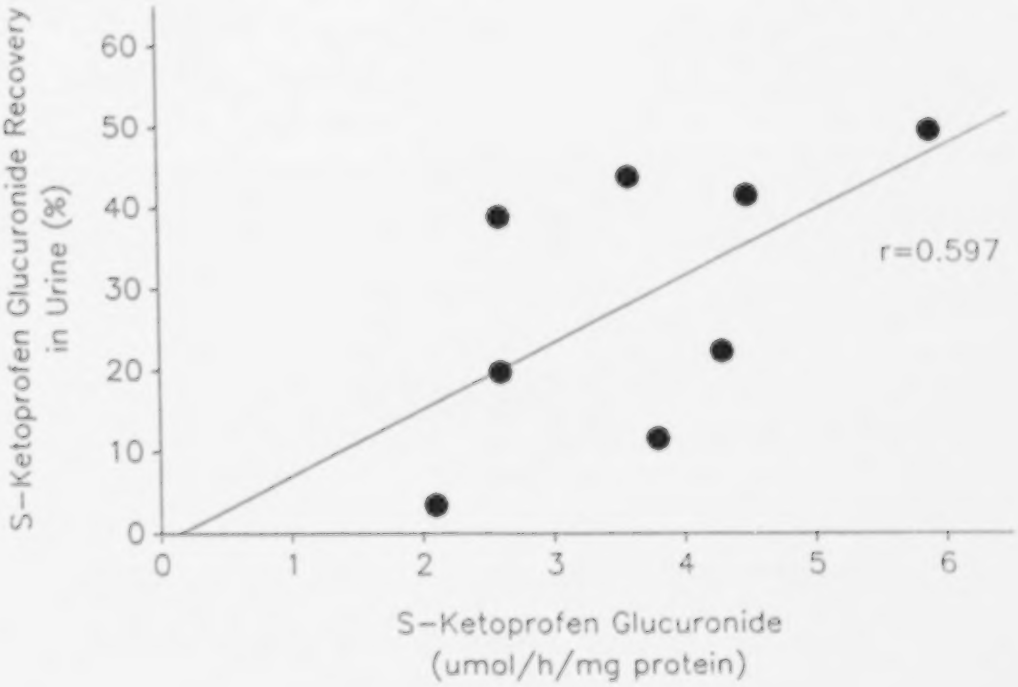

Figure 13. Correlation of mean percent of S-ketoprofen dose as S-ketoprofen glucuronide recovered in 24 h urine collection (n = 4 rats) and mean amount of S-ketoprofen glucuronide formed by kidney microsomes (n = 3 incubations) from gonadectomized and sex hormone treated male and female rats.

3.2.3. Gonadectomy and sex hormones

3.2.3.1. Female rats

No significant differences in mean ΣXu_{urine} of S-ketoprofen glucuronide as percent of S-ketoprofen dose recovered in urine were found between ovarectomized female and control female rats at any time (Figure 14, Table VIII). A significant decrease in the urinary recovery of S-ketoprofen glucuronide in ovarectomized + testosterone treated females (29.1 $\pm$ 14.8%) from both ovarectomized females (48.1 $\pm$ 19.6%) and control females (57.5 $\pm$ 28.5%) was not observed until week 3 (Table VIII). The decrease in urinary excretion of S-ketoprofen glucuronide within the ovarectomized + testosterone treated female group became significantly different from week 1 at week 2 (37.1 $\pm$ 8.5%) and steadily decreased to 13.4 $\pm$ 5.4% of the dose being recovered at week 11 (Figure 14). No significant differences in urinary recoveries of S-ketoprofen glucuronide were found between control and sham-operated females or control and ovarectomized + estradiol treated females at any time. In all cases, gonadectomy or hormonal treatment had no effect on the negligible recovery of R-ketoprofen glucuronide recovered in urine.

3.2.3.2. Male rats

A rapid and significant increase in the ΣXu_{urine} of S-ketoprofen glucuronide recovered in 24 h from urine of castrated males (27.9 $\pm$ 9.0%) and

castrated + estradiol treated males (38.4 ± 15.8%) from that of control males (7.2 ± 3.9%) was evident after only 1 week (Figure 15, Table VIII). In castrated male rats, the amount of S-ketoprofen glucuronide recovered in urine remained high with a significant further increase from week 1 observed at week 2 (43.5 ± 20.3%) (Figure 15). Similarly, in castrated + estradiol treated males, the amount of S-ketoprofen glucuronide recovered remained high with a significant further increase from week 1 evident at week 3 (47.6 ± 21.1%) (Figure 15). At week 11, the amount recovered from urine of castrated + estradiol males (71.9 ± 16.9%) was significantly higher than from castrated males (57.3 ± 16.2%) (Table VIII). No significant differences in urinary recoveries of S-ketoprofen glucuronide were found between control males and sham-operated males at any time. In castrated + testosterone treated males, a trend towards lower S-ketoprofen glucuronide urinary excretion (4.7 ± 2.0 % and 3.5 ± 1.0%) at weeks 1 and 4 when compared with control (7.2 ± 3.9% and 11.6 ± 1.6%) and sham-operated (6.0 ± 1.5% and 7.8 ± 4.9%) males at the same times (Figure 12) was observed, however, values failed to reach statistical significance (Table VII). There was no treatment effect on the small urinary recovery of R-ketoprofen glucuronide in the males, similar to what was seen in the female rats.

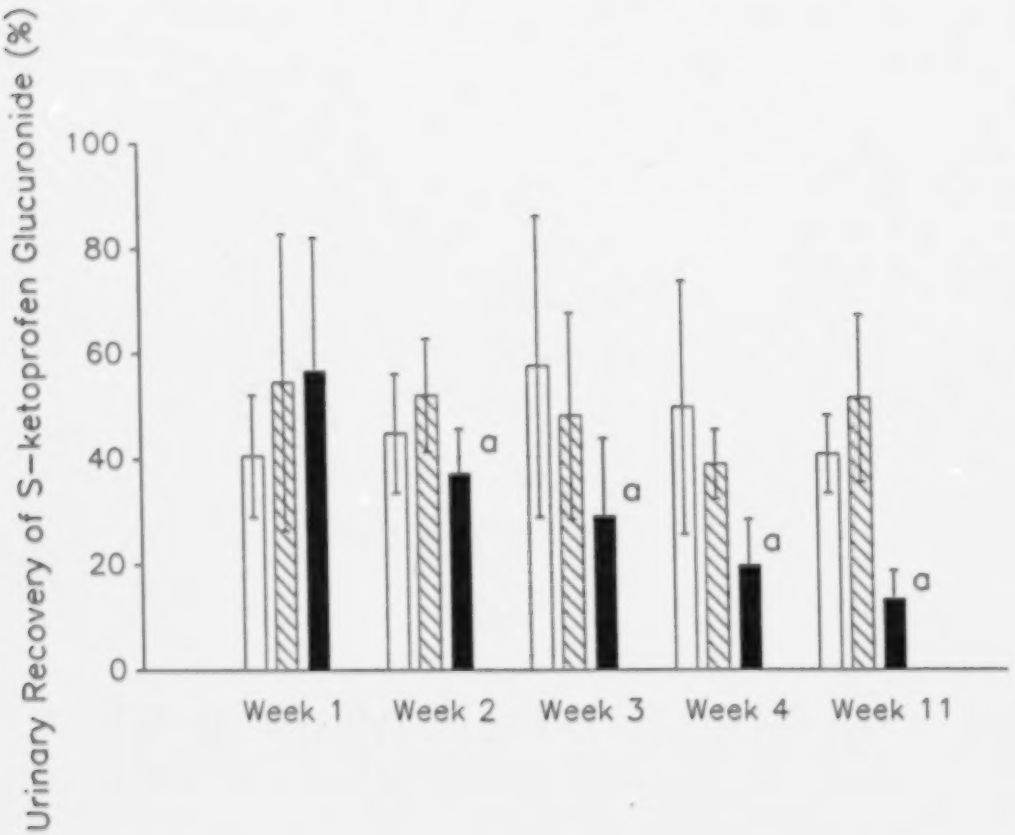

Figure 14. Percent of S-ketoprofen dose as S-ketoprofen glucuronide recovered in 24 h urine collection with time from gonadectomy in control (▫), ovarectomized (▨) and ovarectomized + testosterone (■) treated female rats. Data are presented as the mean ± SD. The letter (a) represents a significant difference from week 1 α = 0.05. Additional statistics given in **Table VIII**.

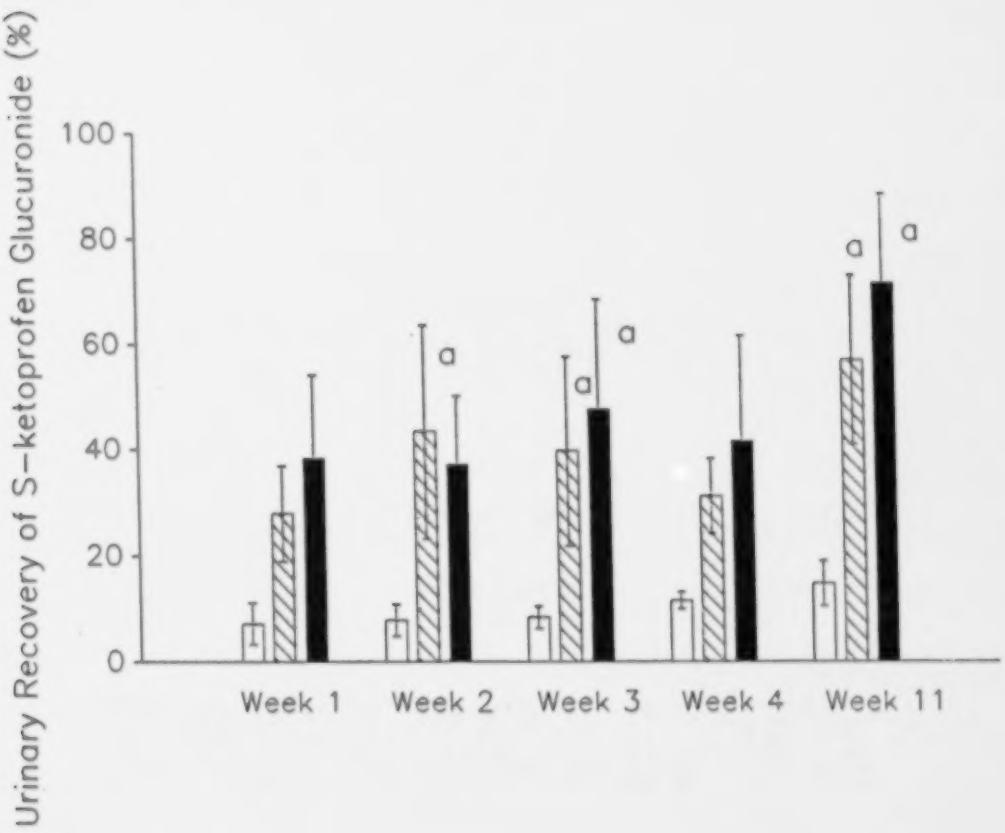

Figure 15. Percent of S-ketoprofen dose as S-ketoprofen glucuronide recovered in 24 h urine collection with time from gonadectomy in control (□), castrated(▨) and castrated + estradiol (■) treated male rats. Data are presented as the mean ± SD. The letter (a) represents a significant difference from week 1 α = 0.05. Additional statistics given in **Table VIII**.

Table VIII. Duncan's Multiple-Range Test[1] results for differences between groups for data in Figures 14 and 15

	Differences between groups					
Week	Figure 14. Female rats			Figure 15. Male rats		
1	CF	OV	OVT	CM	CS	CSE
2	OVT	CF	OV	CM	CSE	CS
3	OVT	OV	CF	CM	CS	CSE
4	OVT	OV	CF	CM	CS	CSE
11	OVT	CF	OV	CM	CS	CSE

[1] Ranked lowest to highest; connecting lines indicate insignificant differences $\alpha = 0.05$

CF, control female; OV, ovarectomy; OVT, ovarectomy + testosterone treated female rats and CM, control male; CS, castration and CSE, castration + estradiol treated male rats.

An interesting observation was the effect of estradiol treatment on weight gain in the male rats. The castrated + estradiol treated male rats showed a significantly lower percent mean weight gain from week 1 than other males or females, including the ovarectomized + estradiol treated female rats (Figure 16).

3.2.4. Plasma protein binding

The mean ± SD percent unbound fractions of R- and S-ketoprofen as a function of total (bound + unbound) plasma concentrations of the enantiomers in male or female rat plasma are depicted in Figure 17. Both ketoprofen enantiomers were found to be extensively bound to plasma and no stereoselectivity or gender difference in the extent of binding of ketoprofen enantiomers was found at any of the concentrations investigated. There was, however, a significant increase in the percent unbound at the enantiomeric concentration of 50 μg/mL for R-ketoprofen in male plasma and at 100 μg/mL for R- and S-ketoprofen in both male and female plasma when compared with lower concentrations (Figure 17). There was no difference in the albumin concentrations of male (3.83 g/dL) or female (3.84 g/dL) plasma.

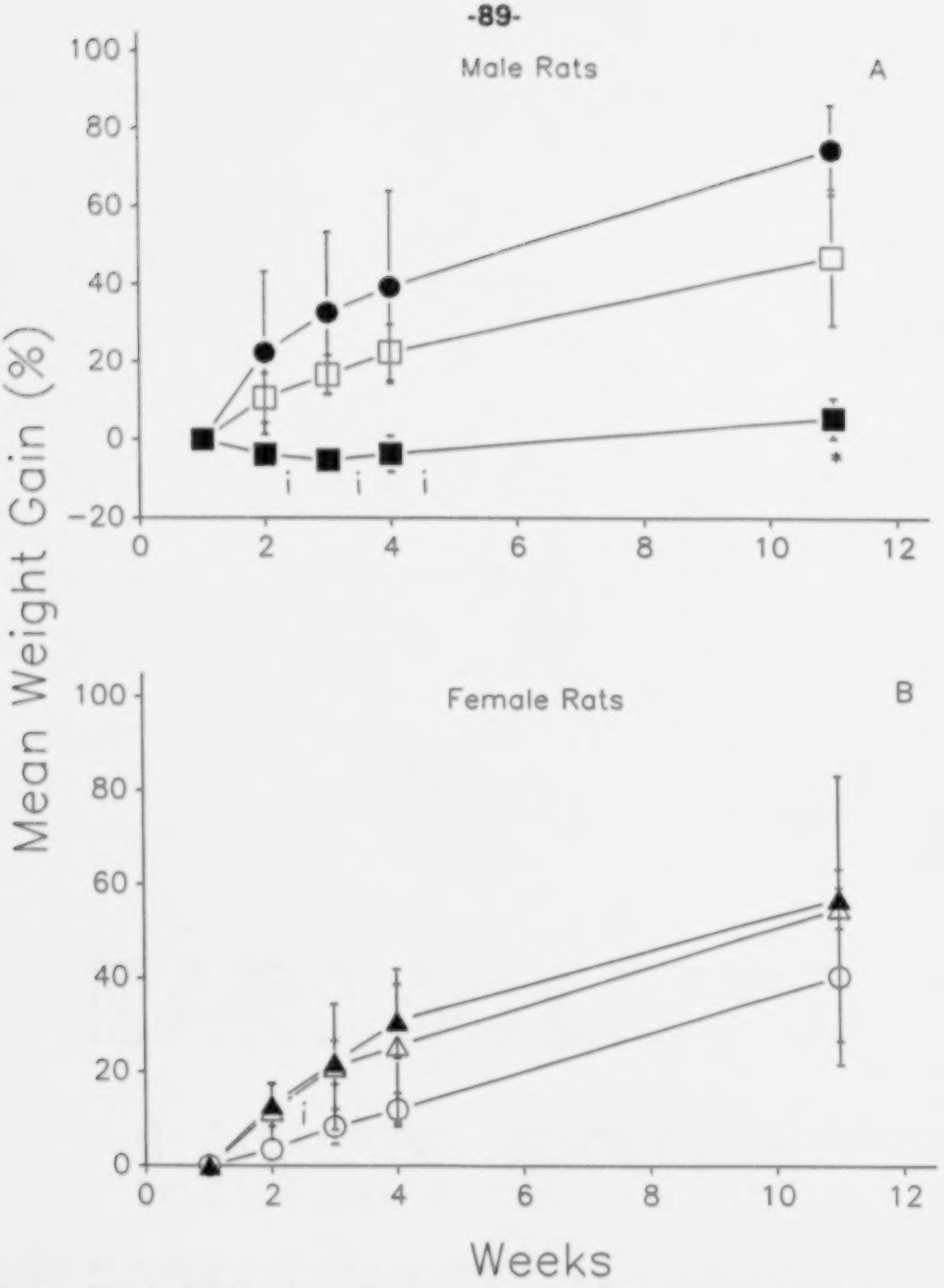

Figure 16. Percent weight gain from week 1 following gonadectomy of (A) control (●), castrated (□) and castrated + estradiol (■) treated male rats and (B) control (○), ovarectomized (▲) and ovarectomized + testosterone treated (▲) female rats. Data are presented as the mean ± SD (n = 4 rats). The letter (i) represents an insignificant difference from week 1 $\alpha = 0.05$.

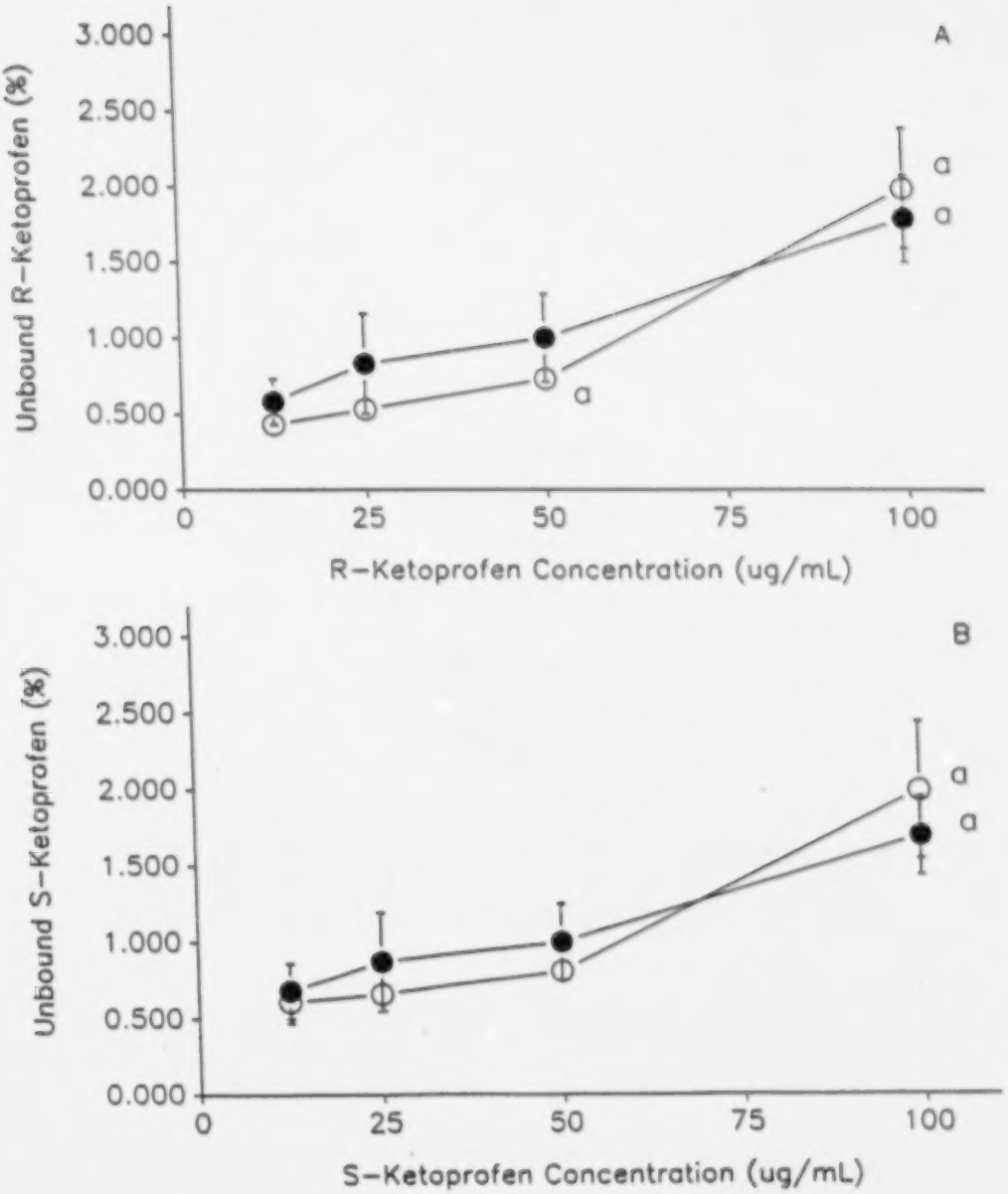

Figure 17. Percent unbound fraction of R-ketoprofen (A) and S-ketoprofen (B) as a function of total (bound + unbound) plasma concentration in male (○) and female (●) rat plasma. Data are presented as the mean ± SD (n = 3). The letter (a) represents a significant difference from lower concentrations α = 0.05.

3.2.5. Intestinal permeability

No significant gender differences were found in the percent ^{51}Cr-EDTA dose excreted in the urine of female or male rats for either baseline values or following the 3 mg/kg oral dose of rac-ketoprofen. The amount of ^{51}Cr-EDTA excreted in the urine following the rac-ketoprofen dose was significantly increased from baseline values in both sexes (Figure 18).

3.3. Interaction of probenecid and ketoprofen in rats

3.3.1. Pharmacokinetics in female rats

Figure 19 is representative of the mean ketoprofen enantiomer and probenecid plasma concentration *vs* time profiles obtained in female rats receiving 10 mg/kg rac-ketoprofen and 0 (saline) or 100 mg/kg probenecid *i.v.* bolus doses. The mean $AUC_{(0\text{-}24h)}$ and percent of ketoprofen enantiomer dose recovered in urine (ΣXu_{urine}) as glucuronidated R- or S-ketoprofen are given in Table IX. Despite the obvious trend of increasing AUC with probenecid dose for both R- and S-ketoprofen, significant differences in AUC from controls were only found for S-ketoprofen with 150 and 200 mg/kg of probenecid (Mean AUCs: 4762 and 4790 mg.h/L respectively) and for R-ketoprofen with 150 mg/kg of probenecid. The results of the statistical analyses are given in Table X. Figure 20 is illustrative of the nonlinear kinetics observed for the range of probenecid doses employed in this study.

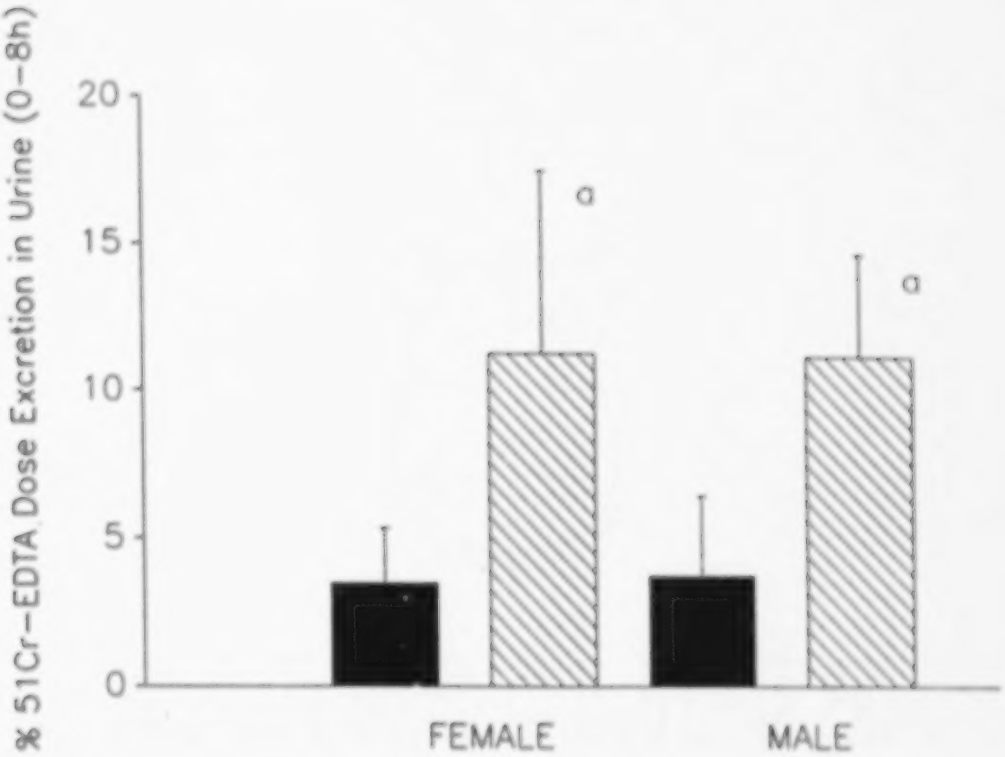

Figure 18. Percent ^{51}Cr-EDTA dose excretion into urine in 8 h in female and male rats. Data are presented as the mean ± SD for baseline (■) and following oral administration of 3 mg/kg rac-ketoprofen (□). The letter (a) represents a significant difference between baseline and ketoprofen treated values (n = 6 rats each) α = 0.05.

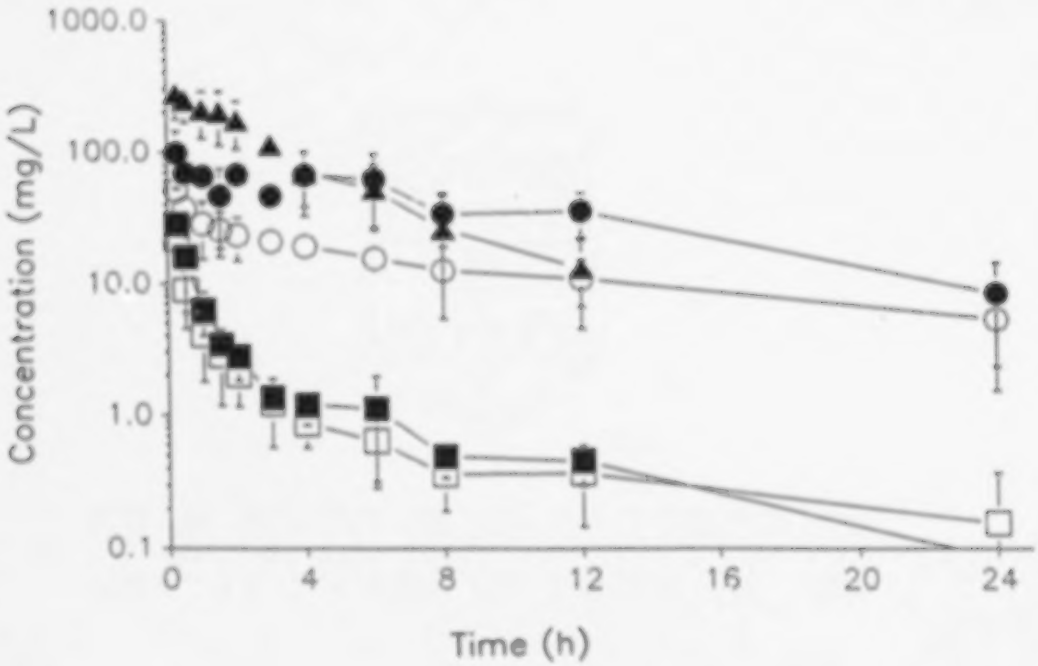

Figure 19. Plasma concentration *vs* time profiles for S-ketoprofen (○), S-ketoprofen with probenecid (●), R-ketoprofen (□), R-ketoprofen with probenecid (■) and probenecid (▲) following *i.v.* administration of 10 mg/kg rac-ketoprofen and 0 (saline) or 100 mg/kg probenecid in female rats. Data are presented as the mean ± SD (n = 3 rats).

Table IX. Plasma and urine pharmacokinetic indices after administration of 10 mg/kg *i.v.* rac-ketoprofen and various doses of probenecid in female rats. Data are presented as the mean ± (SD)

Group	Probenecid			S-ketoprofen			R-ketoprofen		
	Dose $_{mg/kg}$	$AUC_{0.5\text{-}24.5h}$	No. of Rats	$AUC_{0\text{-}24h}$	ΣXu_{urine} %	CL_{renal} mL/h	$AUC_{0\text{-}24h}$	ΣXu_{urine} %	CL_{renal} mL/h
A	0	0 (0)	6	320 (89)	16.8 (6.0)	0.78 (0.31)	26 (7)	2.5 (0.9)	1.27 (0.30)
B	25	49 (35)	3	356 (141)	23.0 (12.1)	0.72 (0.21)	27 (7)	1.6 (0.5)	0.74 (0.14)
C	50	341 (271)	3	719 (153)	30.5 (12.5)	0.67 (0.41)	54 (7)	1.8 (1.0)	0.48 (0.28)
D	100	1205 (230)	3	905 (254)	21.1 (5.4)	0.35 (0.15)	37 (8)	0.8 (0.1)	0.31 (0.07)
E	150	4762 (1238)	3	1705 (552)	35.0 (4.5)	0.27 (0.02)	81 (11)	1.4 (0.7)	0.16 (0.06)
F	175[1]	3231 4056	2	814 797	12.2 12.7	0.20 0.22	34 49	0.6 0.3	0.25 0.08
G	200	4790 (1459)	3	1258 (519)	18.5 (6.7)	0.54 (0.35)	51 (32)	0.6 (0.2)	0.24 (0.15)

[1] Because of small sample size, individual values are given and excluded from the statistical analysis. See Table X for statistical analysis.

Table X. Duncan's Multiple Range Test[1] results for data in Table IX

Indices	S-ketoprofen	R-ketoprofen
$AUC_{(0\text{-}24h)}$	A B C D G E	A B D G C E
ΣXu_{urine} (%)	A G B D C E	G D E B C A
CL_{ucorr} (mL/h)	E D G C B A	E G D C B A

[1] Ranked lowest to highest; connecting lines indicate insignificant differences $\alpha = 0.05$

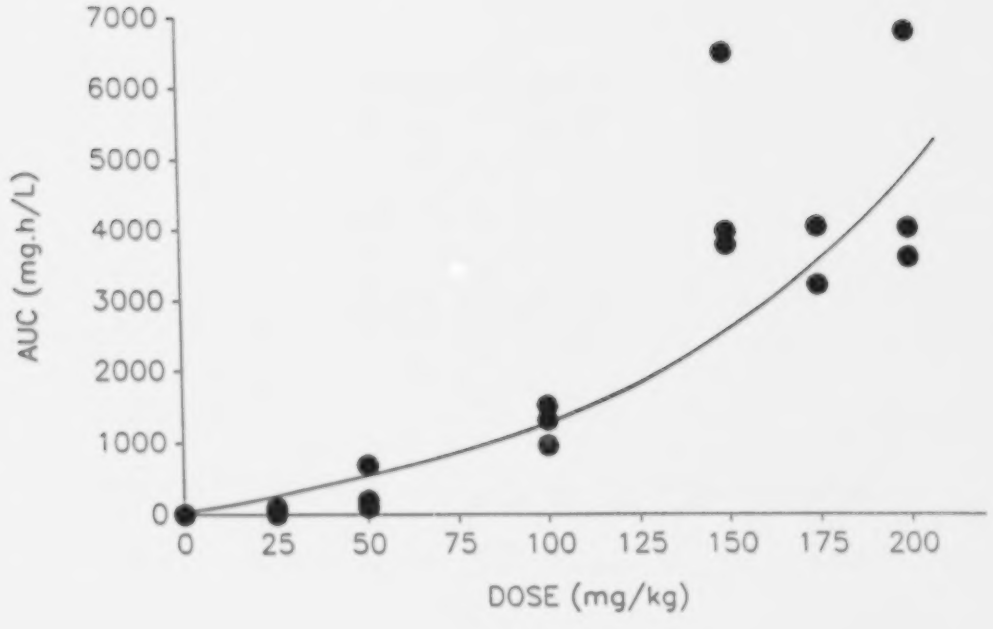

Figure 20. $AUC_{(0.5\text{-}24.5h)}$ *vs* dose relationship of probenecid after administration of 0-200 mg/kg probenecid and a constant dose of 10 mg/kg rac-ketoprofen *i.v.* in female rats.

The ΣXu_{urine} of R-ketoprofen glucuronide decreased significantly from controls following doses of 100 and 200 mg/kg probenecid which corresponds to AUCs of 1205 and 4790 mg.h/L of probenecid. The ΣXu_{urine} of S-ketoprofen glucuronide did not differ significantly from controls with the exception of a significant increase following 150 mg/kg (4762 mg.h/L) probenecid. The amount of non-glucuronidated R- or S-ketoprofen appearing in urine in either control or probenecid dosed rats was negligible.

Depicted in Table XI are correlations resulting from pooling the data of all rats at each sample time and plotting plasma levels of R- and S-ketoprofen *vs* those of probenecid. Generally strong positive correlations were found for all sampling times with the exception of the 24 h samples as by this time there was no longer any detectable probenecid in plasma at any dose. By 4.5 h post-probenecid dosing (4.0 h post-ketoprofen dosing) there was no longer any probenecid detectable in plasma of the 25 mg/kg probenecid dosed rats. In order to include probenecid plasma data from all probenecid dosed rats, we examined the correlation of $AUC_{(0.5\text{-}4.0h)}$ of R-ketoprofen ($r = 0.716$) and S-ketoprofen ($r = 0.899$) with probenecid $AUC_{(0.5\text{-}4.5h)}$ and similarly found strong relationships.

Overall the strongest correlation (Figure 21) between probenecid plasma concentrations and those of R-ketoprofen ($r = 0.708$) and S-ketoprofen ($r = 0.913$) were found at 2.0 h and 1.5 h post-ketoprofen dosing (2.5 and 2.0

Table XI. Correlation[1] of R-ketoprofen and S-ketoprofen plasma concentrations with probenecid plasma concentrations at individual sampling times in female rats

Time (h)	S-ketoprofen (r)	R-ketoprofen (r)
0.25	0.669	0.682
0.50	0.827	0.657
1.00	0.856	0.654
1.50	0.913	0.678
2.00	0.892	0.708
3.00	0.902	0.659
4.00	0.838	0.661
6.00	0.790	0.547
9.00	0.831	0.670
12.00	0.781	0.616

[1] All values were statistically significant

Table XII. S:R-ketoprofen ratios[1] in plasma and urine for each probenecid dose and MRT in female rats. Data are presented as the mean ± (SD).

Probenecid Dose (mg/kg)	Probenecid MRT (h)	S:R $AUC_{(0\text{-}24h)}$	S:R ΣXu_{urine}
A. 0	0 (0)	12.1 (2.3)	6.8 (0.7)
B. 25	0.9 (0.3)	13.2 (3.8)	12.7 (4.5)
C. 50	2.3 (1.0)	13.3 (2.0)	19.4 (7.4)
D. 100	4.4 (1.3)	24.5 (5.4)	26.0 (7.9)
E. 150	4.0 (0.3)	20.7 (3.8)	30.2 (11.4)
F. 175	5.8 (0.7)	23.9 (6.3)	30.7 (10.3)
G. 200	6.0 (1.6)	27.6 (5.0)	36.4 (12.2)

[1] See **Table XIII** for statistical analysis

Table XIII. Duncan's Multiple Range Test[1] results for data in Table XII

Indices	S:R-ketoprofen ratios
$AUC_{(0\text{-}24\ h)}$	A B C E F D G
ΣXu_{urine}	A B C D E F G

[1] Ranked lowest to highest; connecting lines indicate insignificant differences $\alpha = 0.05$

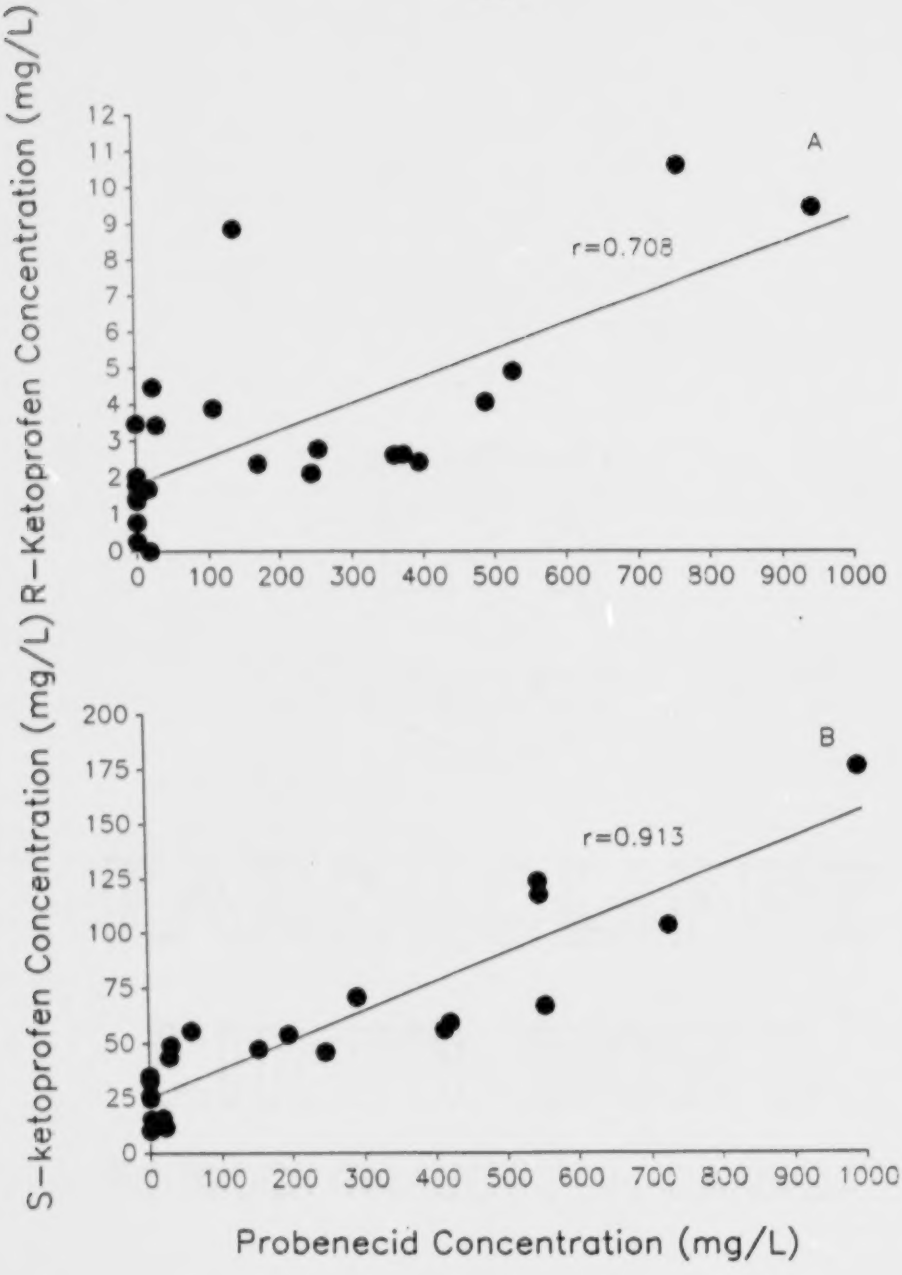

Figure 21. Correlation of R-ketoprofen (A) or S-ketoprofen (B) and probenecid plasma concentrations at 2.0 h (A) or 1.5 h (B) sampling times following administration of 0-200 mg/kg probenecid and 10 mg/kg rac-ketoprofen *i.v.* doses in female rats.

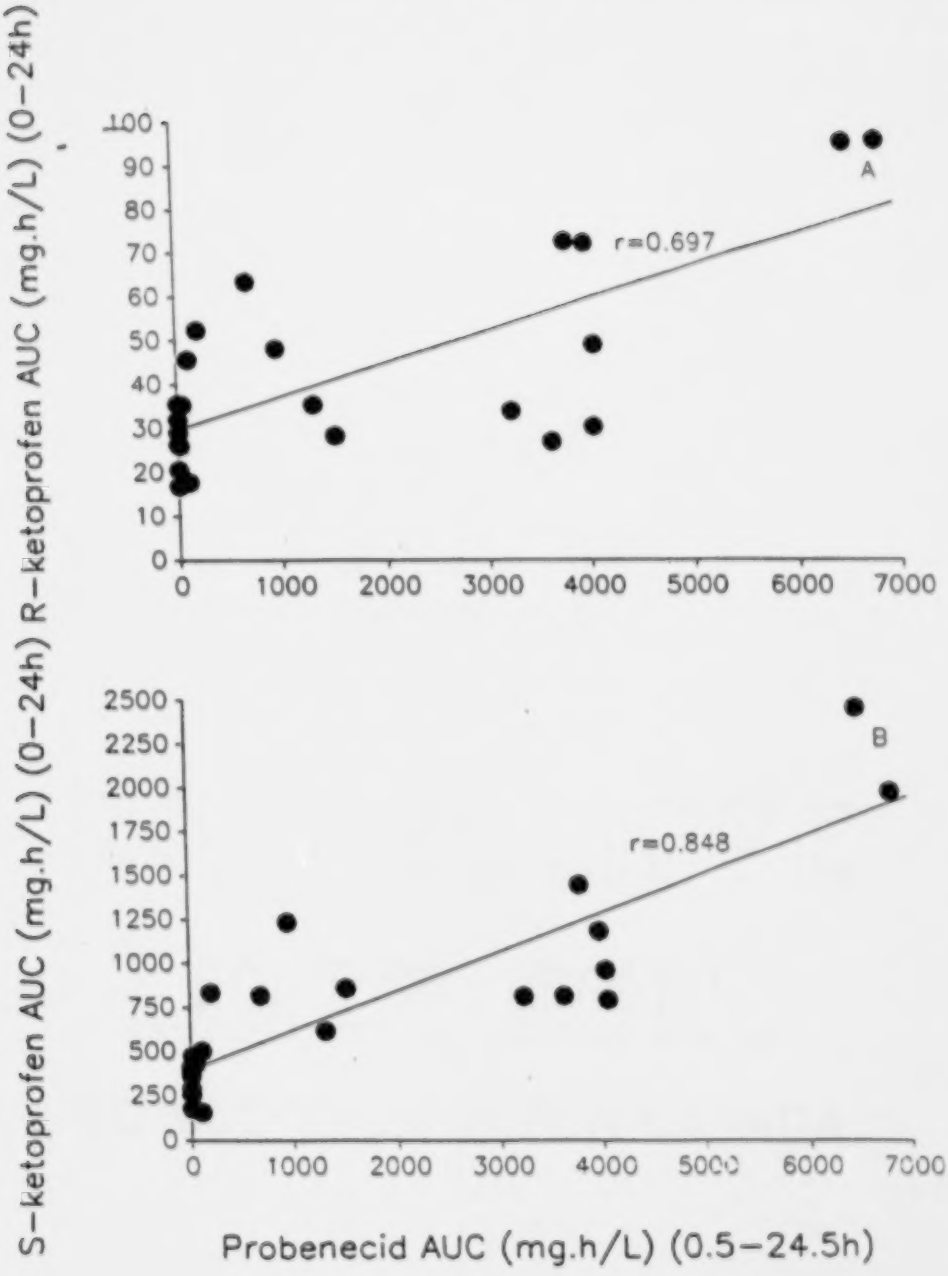

Figure 22. Correlation of $AUC_{(0-24h)}$ of R-ketoprofen (A) or S-ketoprofen (B) and $AUC_{(0.5-24.5h)}$ of probenecid after 0-200 mg/kg probenecid and 10 mg/kg rac-ketoprofen *i.v.* doses in female rats.

h post-probenecid dosing), respectively. Illustrated in Figure 22 is the relationship between $AUC_{(0\text{-}24h)}$ of R-ketoprofen ($r = 0.697$) and S-ketoprofen ($r = 0.848$) with probenecid $AUC_{(0.5\text{-}24.5h)}$.

A progressive rise in S:R ketoprofen ratios of both $AUC_{(0\text{-}24h)}$ from 12.1 to 27.6 and ΣXu_{urine} of the enantiomer glucuronides from 6.8 to 36.4 with increasing probenecid dose (0-200 mg/kg) is depicted in Table XII. Significant positive correlations were found for S:R ketoprofen ratios and probenecid dose (mg/kg) (AUC: $r = 0.683$; ΣXu_{urine}: $r = 0.779$) as well as probenecid MRT (AUC: $r = 0.641$; ΣXu_{urine}: $r = 0.678$). Significant differences in S:R ketoprofen AUC ratios between control and probenecid treated rats were found following 200 mg/kg probenecid and for S:R ketoprofen glucuronides ΣXu_{urine} following doses of 100, 150 and 200 mg/kg probenecid.

3.3.2. Pharmacokinetics in male rats

Illustrated in Figure 23 is the mean ketoprofen enantiomer and probenecid plasma concentration *vs* time profiles obtained in male rats receiving 0 (saline) or 100 mg/kg probenecid *i.v.* bolus doses. A significant increase in S-ketoprofen AUC in the presence of probenecid in the male rat was observed (Table XIV). The extent of interaction of probenecid with S-ketoprofen, however, appears to be gender dependent as previously an

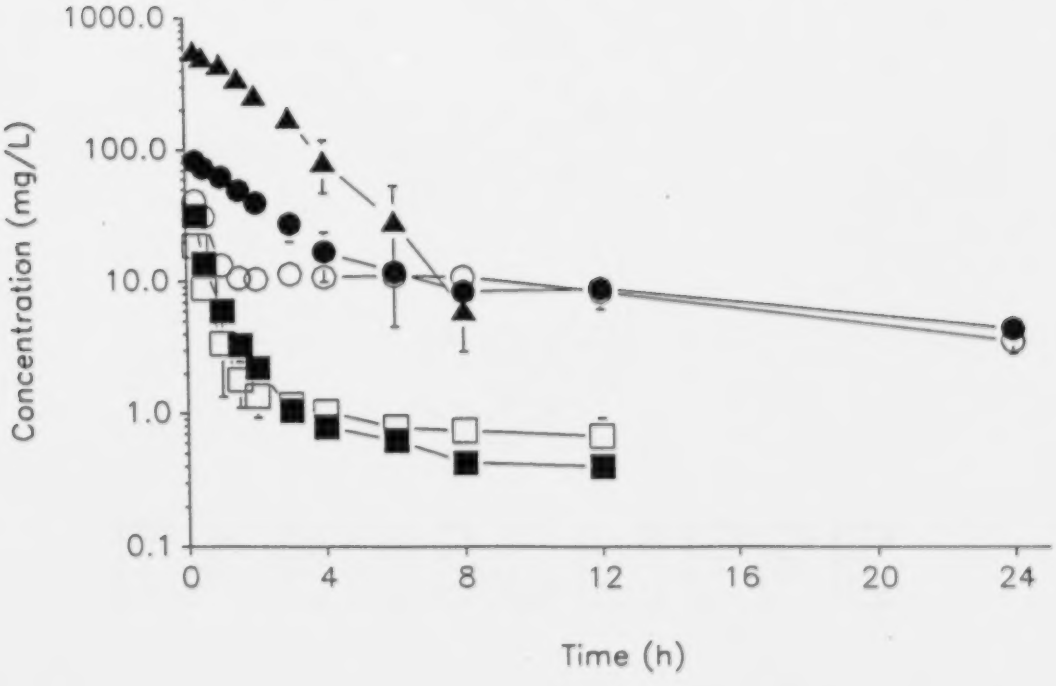

Figure 23. Plasma concentration *vs* time profiles for S-ketoprofen (○), S-ketoprofen with probenecid (●), R-ketoprofen (□), R-ketoprofen with probenecid (■) and probenecid (▲) following *i.v.* administration of 10 mg/kg rac-ketoprofen and 0 (saline) or 100 mg/kg probenecid in male rats. Data are presented as the mean ± SD (n = 4 rats).

Table XIV. Plasma and urine pharmacokinetic indices after administration of 10 mg/kg *i.v.* rac-ketoprofen and 0 (saline) or 100 mg/kg of probenecid in male rats. Data are presented as the mean ± (SD).

Probenecid			S-Ketoprofen			R-ketoprofen		
Dose, mg/kg	AUC (0.5-24.5)	No. of Rats	AUC (0-24)	ΣXu% (0-24)	CL_{ucorr} mL/h	AUC (0-24)	ΣXu% (0-24)	CL_{ucorr} mL/h
0	0	4	200 (97)	11.0 (4.5)	0.55 (0.25)	25 (9)	2.3 (0.54)	1.7 (1.2)
100	1288 (156)	4	375* (45)	7.1 (2.4)	0.28 (0.13)	43 (19)	0.51* (0.14)	0.30* (0.14)

*Significantly different from control (α = 0.05).

increase of 182% in the AUC of S-ketoprofen in the presence of 100 mg/kg *i.v.* probenecid in female rats whereas only an 88% increase in S-ketoprofen AUC was seen in male rats.

The mean AUC of probenecid in the male rat (Table XIV) was very similar to that previously obtained in the female rat (Table IX) following 100 mg/kg of probenecid, thus, gender differences in probenecid disposition appears unlikely.

The amount of non-glucuronidated R- or S-ketoprofen recovered in urine was negligible. The ΣXu_{urine} was significantly reduced with probenecid for R- but not for S-ketoprofen glucuronide (Table XIV), similar to female rats (Table IX).

3.3.3. Role in *in vivo* disposition of ketoprofen glucuronide conjugates

3.3.3.1. Intact rats

The mean plasma $AUC_{(0\text{-}24h)}$ of S-ketoprofen glucuronide was significantly higher when given with probenecid (38.3 ± 10.6 mg.h/L) than without probenecid (11.7 ± 4.1 mg.h/L) (Figure 24). Accordingly, the CL_{TB} of S-ketoprofen glucuronide was significantly lower in the presence of probenecid than in its absence (0.22 ± 0.11 *vs* 0.87 ± 0.16 mL/min, respectively). Concomitant probenecid administration resulted in an increase in mean plasma $AUC_{(0\text{-}24h)}$ of R-ketoprofen glucuronide from 4.8 ± 3.0 mg.h/L to 8.1 ± 4.2 mg.h/L, however, the values failed to reach statistical significance

(Figure 24). A trend towards lower ΣXu_{urine} in 24 h of the enantiomer glucuronides was seen in the presence of probenecid than in its absence (R: 18.3 ± 3.5% *vs* 24.4 ± 7.9% and S: 42.7 ± 9.9% *vs* 59.0 ± 20.6%, respectively); however, values were not statistically different. The majority of the amount of enantiomer glucuronide excreted in urine was excreted in the first 0-3 h following the dose of the glucuronides. The percent of enantiomer glucuronide excreted from 0-3 h as a percentage of the total amount excreted in 24 h was R: 93.0 ± 7.23% and S: 39.18 ± 9.56% (with probenecid) and R: 86.9 ± 7.86% and S: 48.10 ± 10.98% (without probenecid). The values were not statistically different.

The mean plasma $AUC_{(0\text{-}24h)}$ of non-glucuronidated (deconjugated) R- and S-ketoprofen were significantly higher when given with probenecid (R: 14.1 ± 2.9; S: 254.1 ± 16.7 mg.h/L) than without (R: 6.3 ±2.1; S: ± 148.7 ± 32.0 mg.h/L) (Figure 25). The negligible recovery in urine of non-glucuronidated R- or S-ketoprofen was not affected by the presence of probenecid.

3.3.3.2. Bile-duct cannulated rats

A trend towards higher plasma $AUC_{(0\text{-}3h)}$ of both ketoprofen enantiomer glucuronides with probenecid (R: 2.9 ± 0.7; S: 3.9 ± 1.0 mg.h/L) than without probenecid (R: 1.1 ± 0.2; S: 2.1 ± 1.4 mg.h/L) was seen but values did not reach statistical significance. There was; however, a

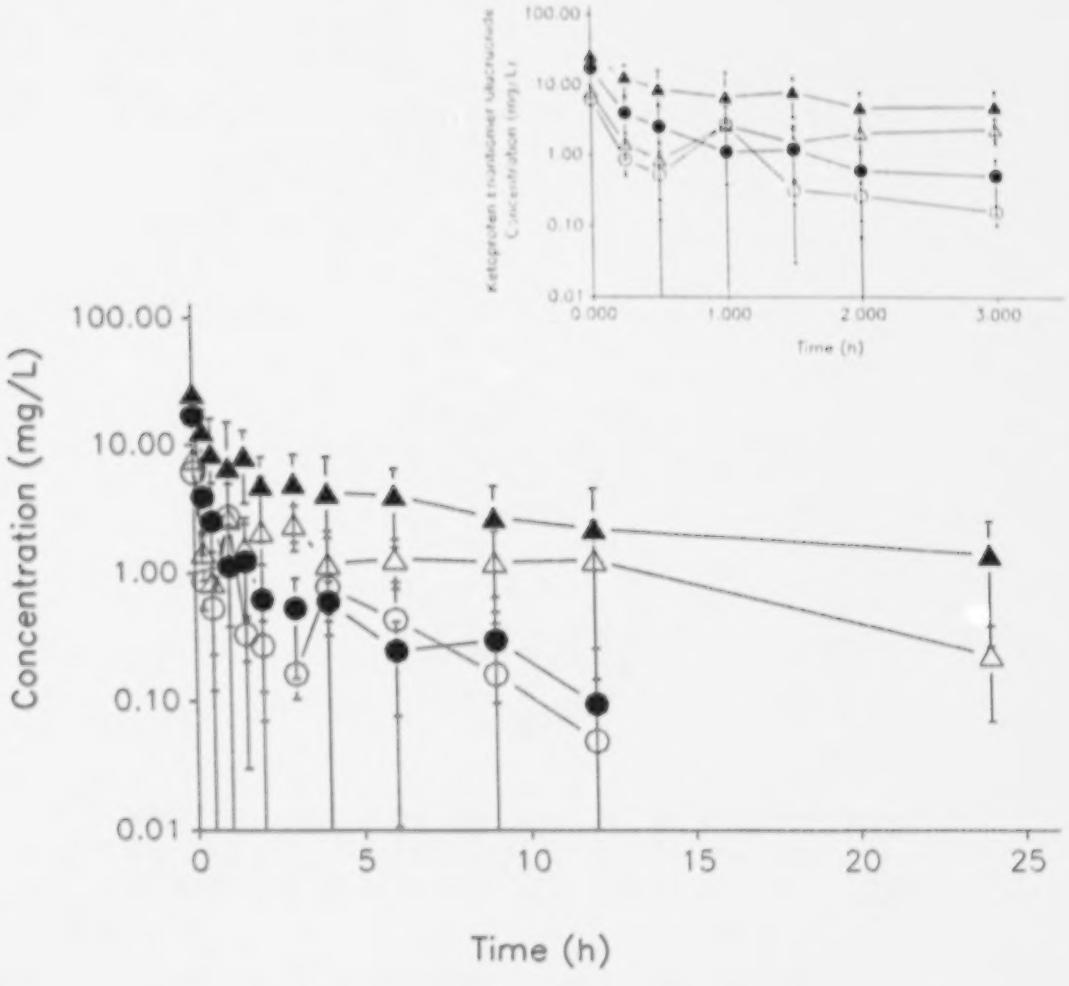

Figure 24. Plasma concentration *vs* time profiles for glucuronidated R-ketoprofen (○) and S-ketoprofen (▲) without probenecid and glucuronidated R-ketoprofen (●) and S-ketoprofen (▲) with probenecid after administration of ≈ 1 mg/kg R- and S-ketoprofen glucuronides and 0 (saline) or 100 mg/kg probenecid *i.v.* in female rats. Data are presented as the mean ± SD (n = 4 rats). Insert is a magnification of the first 3 h.

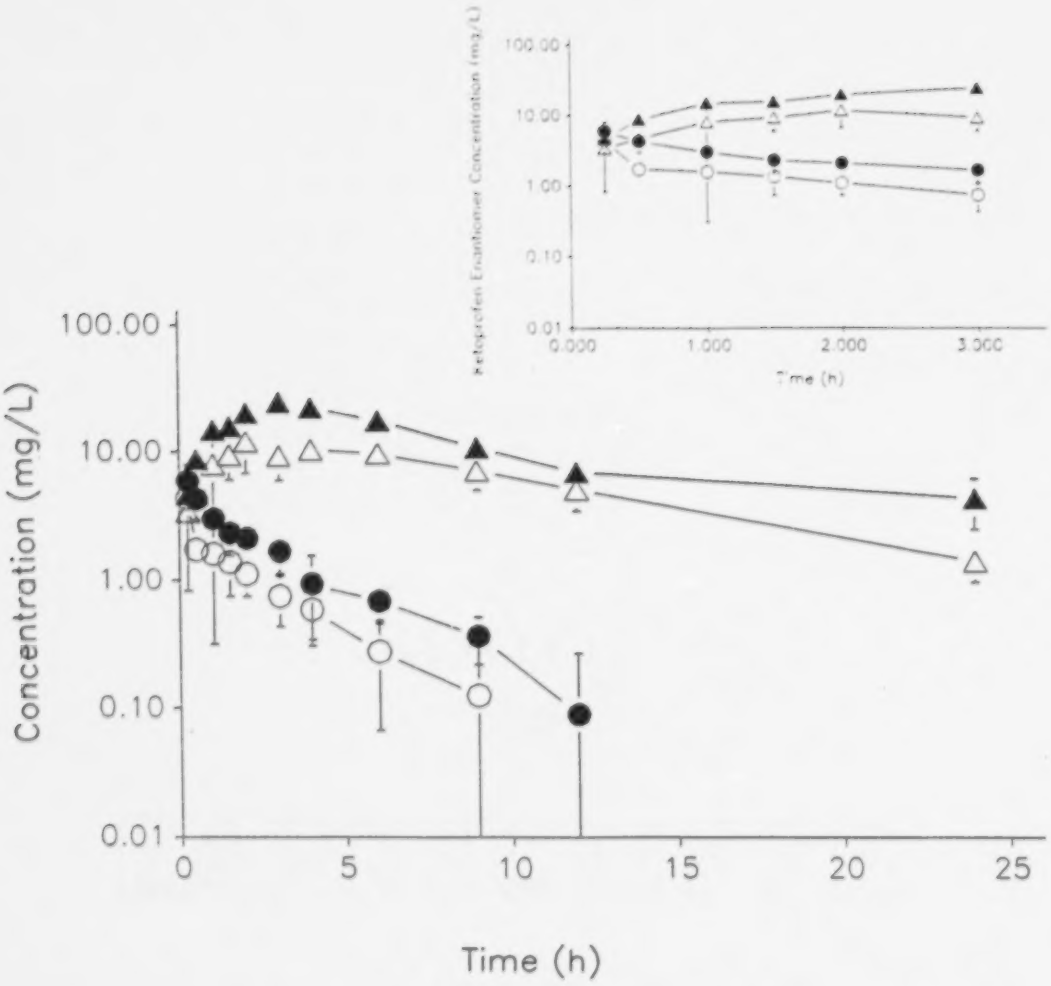

Figure 25. Plasma concentration *vs* time profiles for non-glucuronidated R-ketoprofen (○) and S-ketoprofen (▲) without probenecid and non-glucuronidated R-ketoprofen (●) and S-ketoprofen with probenecid (▲) after administration of ≈ 1 mg/kg R- and S-ketoprofen glucuronides and 0 (saline) or 100 mg/kg probenecid in female rats. Data are presented as the mean ± SD (n = 4 rats). Insert is a magnification of the first 3 h.

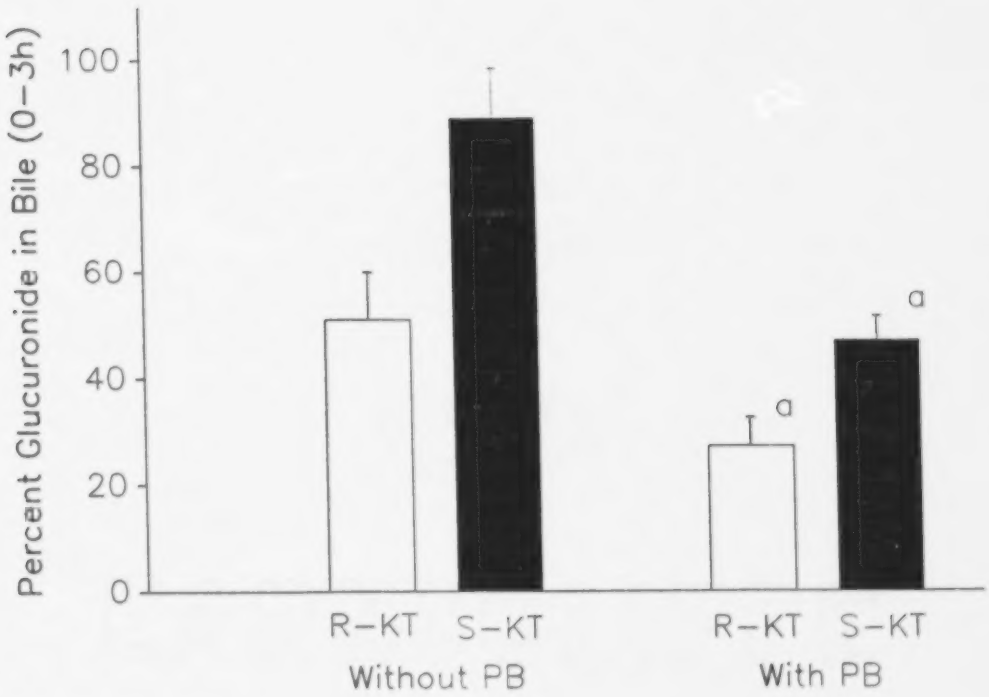

Figure 26. Percent glucuronidated R-ketoprofen (R-KT) (□) or S-ketoprofen (S-KT) (■) with or without probenecid (PB) recovered from 0-3 h collection of bile in female rats after administration of ≈ 1 mg/kg R- and S-ketoprofen glucuronides and 0 (saline) or 100 mg/kg of probenecid *i.v.* Data are presented as the mean ± SD (n = 3 rats). The letter (a) represents a significant difference from without probenecid $\alpha = 0.05$.

significant reduction in the ΣXu_{bile} of R-ketoprofen glucuronide and S-ketoprofen glucuronide as percent of enantiomer glucuronide dose when given with probenecid (R: 27.2 ± 5.4; S: 47.0 ± 4.7%) than without probenecid (R: 51.0 ± 8.9; S: 89.0 ± 9.41%) (Figure 26). The ΣXu_{urine} was not calculated because the urine output from 0-3 h in these rats was negligible.

3.3.4. Role in *in vitro* glucuronidation of ketoprofen

3.3.4.1. Liver microsomal activity

A significant reduction in the amount of both R-ketoprofen glucuronide and S-ketoprofen glucuronide formed by liver microsomes was observed when probenecid was present in the incubation media at any of the concentrations investigated (Figure 27). It was apparent that as the concentration of probenecid increased, the amount of R-ketoprofen glucuronide or S-ketoprofen glucuronide formed decreased. When 1000 μM probenecid was present in the reaction mixture, the amount of ketoprofen enantiomer glucuronides formed (R: 8.2 ± 0.9; S: 8.6 ± 0.1 μmol/h/mg protein) was approximately 50% lower than when probenecid was absent (R: 16.9 ± 1.6; S: 13.9 ± 1.2 μmol/h/mg protein). The amount of R- or S-ketoprofen glucuronides formed in the presence of 1000 μM probenecid was significantly lower than the amounts formed when 200 μM or 40 μM concentrations of probenecid were present (Figure 27).

3.3.4.2. Kidney microsomal activity

A trend towards a reduction in the amount of both R-ketoprofen glucuronide and S-ketoprofen glucuronide formed by kidney microsomes at all of the concentrations of probenecid employed in this study was observed when compared with control microsomes (Figure 28). None of the values; however, reached statistical significance most likely due to the large variablity present.

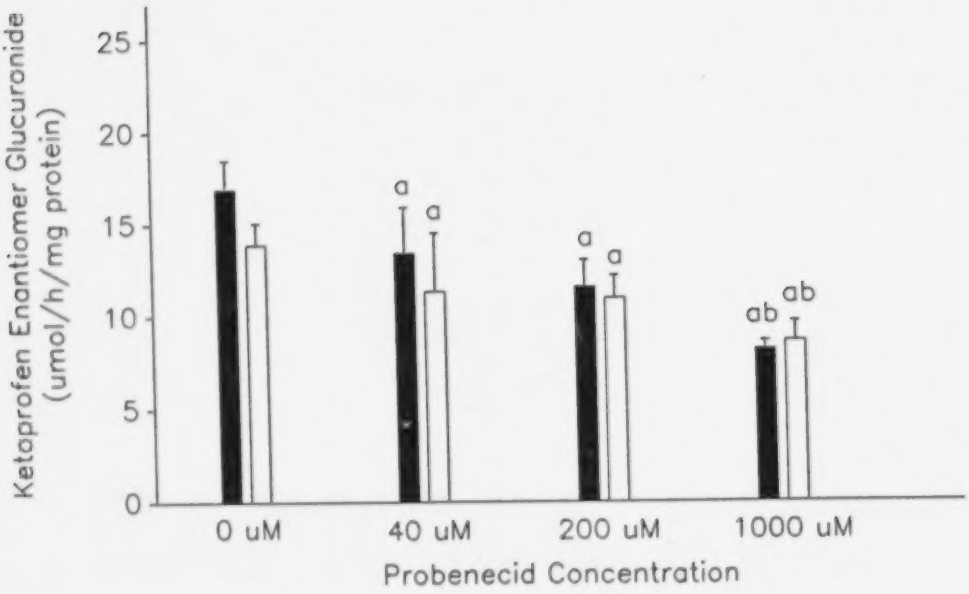

Figure 27. Glucuronidation of R-ketoprofen (■) and S-ketoprofen (□) by liver microsomes in the presence of various concentrations of probenecid. Data are presented as the mean ± SD (n = 3 incubations). The letters (a) represent a significant difference from 0 μM probenecid concentration and (b) represents a significant difference from 0, 40 and 200 μM probenecid concentrations.

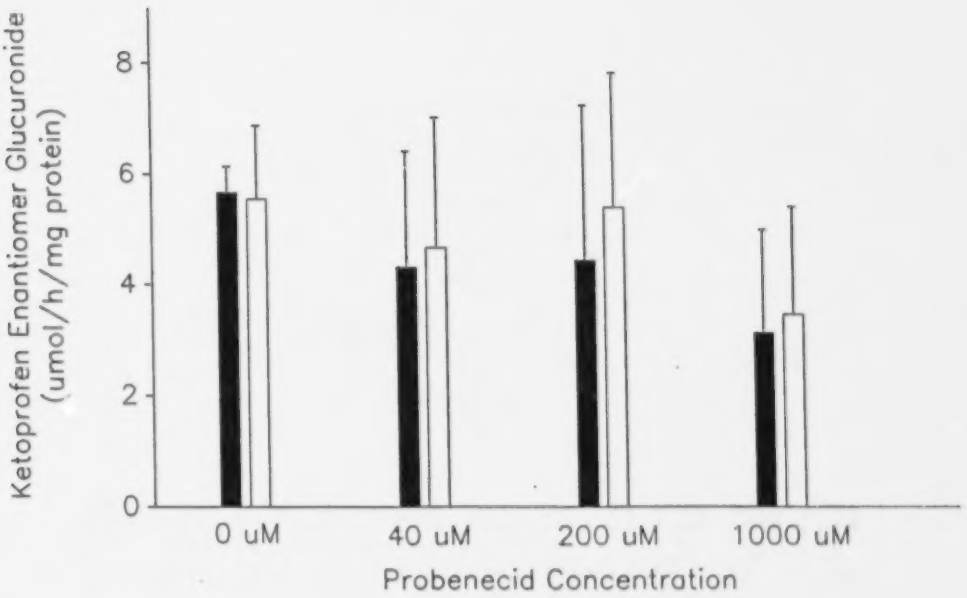

Figure 28. Glucuronidation of R-ketoprofen (■) and S-ketoprofen (□) by kidney microsomes in the presence of various concentrations of probenecid. Data are presented as the mean ± SD (n = 3 incubations). There were no significant differences at any probenecid concentration.

4. DISCUSSION

4.1. Gender dimorphism in the stereoselective pharmacokinetics of ketoprofen in rats

4.1.1. Disposition in rats

The predominant enantiomer in plasma, urine or bile in rats was S-ketoprofen which indicates marked stereoselectivity in the pharmacokinetics of ketoprofen regardless of sex. In both intact and bile-duct cannulated female rats, significantly higher plasma AUC and slower CL_{TB} of both R- and S-ketoprofen were found than in male rats (Table VI).

The mean AUC values for both ketoprofen enantiomers in male rats (Table VI) were surprisingly lower than those previously reported (Table II) following an *i.v.* dose of 10 mg/kg rac-ketoprofen. The discrepancy may be attributed, in part, to the fact that the AUCs reported in Table VI encompass concentrations from 0-24 h after the dose whereas the values reported in Table II are those from 0-∞. Furthermore, the latter mean AUCs were calculated from the data of 5 rats (S-ketoprofen $AUC_{(0-\infty)}$ ranged from 362-1362 mg.h/L) whereas our calculations were based on data from 9 rats (S-ketoprofen $AUC_{(0-24h)}$ ranged from 185-731 mg.h/L). The sum of R- and S-ketoprofen AUC values of female rats given in Table VI are in close agreement with the nonstereospecific data of Brugerolle and Bouvenot (1991), following 10 mg/kg *i.v.* doses of rac-ketoprofen.

There were marked differences in the appearance of S-ketoprofen plasma concentration - time profiles between female and male rats either in intact or bile-duct cannulated animals (Figures 5 & 7). In intact male rats, a pattern of a pronounced drop followed by rise in concentration of S-ketoprofen at 4 h post dose was observed (Figure 5B). This was thought to be suggestive of substantial enterohepatic recirculation, as has previously been reported in male rats (Jamali and Foster, 1988), and was later confirmed by the 5.5 fold decrease in S-ketoprofen AUC following bile-duct cannulation in male rats (Table VI). In intact female rats, this pattern was either much less prominent or absent which may be indicative of relatively less enterohepatic recirculation (Figure 5A). This is supported by the observation of only a 1.5 fold reduction in S-ketoprofen AUC in bile-duct cannulated females when compared with intact female rats (Table VI). As a result, the slower CL_{TB} in the female rat was initially thought to result from a gender dimorphism in biliary clearance, primarily affecting the active S-ketoprofen enantiomer.

The assumption of a sex difference in biliary clearance was further supported by the finding that CL_{bconj} of S-ketoprofen was significantly greater in the male as opposed to female rat despite almost equal overall cumulative biliary recovery of ketoprofen from 0-9 h in both sexes (Figure 8). In addition, the elimination $t_{1/2}$s of both R and S-ketoprofen were significantly shorter in bile-duct cannulated males when compared with female rats, most likely reflecting the efficient biliary clearance in the male rats (Table VI).

The pattern of biliary excretion (Fleck and Braunlich, 1984) and rat bile acid composition (Vonk *et al.*, 1978) may be under hormonal control. The pharmacokinetic parameters of S-ketoprofen in the male when compared with those in the female rat may not only be a reflection of the efficiency of recirculation in the male, but also perhaps, of the amount of glucuronide available for fecal elimination or for deconjugation and reabsorption. As absorption is expected to be a passive and efficient process, the deconjugated S-ketoprofen is expected to be reabsorbed almost completely and so a large portion of the S-ketoprofen AUC in intact male rats is due to recirculated drug. In fact, the fraction of S-ketoprofen AUC attributed to enterohepatic recirculation was much greater in the male (70%) than female (30%) rat. Cleavage to aglycone in the intestine is reported to be influenced by microflora, intestinal transit time, luminal contents and bile duct permeability (Moseley, 1991), all of which may differ due to gender. Hence, it is likely that a gender difference in some component of biliary clearance is, in part, responsible for the dimorphism observed in the disposition of ketoprofen enantiomers in the rat.

In bile-duct cannulated male rats, significantly lower plasma AUC of R-ketoprofen when compared with intact males was found; however, CL_{TB} of R-ketoprofen was not statistically different between them (Table VI). In bile-duct cannulated female rats a larger mean R-ketoprofen plasma AUC was observed when compared with intact females although neither AUC or CL_{TB}

of R-ketoprofen was statistically different between them (Table VI). The higher R-ketoprofen AUC was unexpected and due to the high values from a single rat. This may be due to error in sample collection as the AUC of S-ketoprofen was also high in this rat compared with others, or is perhaps explained by error in quantitation of R-ketoprofen as plasma levels were at or below the detection limit of the assay in many rats in contrast to the high levels of S-ketoprofen.

There was no significant gender difference in either cumulative biliary or urinary recovery of R-ketoprofen in these rats which is not surprising since the main route of elimination of R-ketoprofen is through bioinversion to the S-antipode (Foster and Jamali, 1988) and marked stereoselectivity was seen in both sexes.

The substantially higher recovery (ΣXu_{urine} and CL_{uconj}) of S-ketoprofen glucuronide in urine of females as compared to male rats (Figure 6) was unexpected due to overall lower CL_{TB} in the female rat (Table VI). It follows that the greater recovery of the glucuronide in urine might lead one to assume more extensive glucuronidation in the female than male rat. This, however, is contrary to the general observation that *in vitro* conjugating activity of male rat liver is substantially higher than female rat liver (Mulder, 1986; Muller-Oerlinghausen and Kunzel, 1968). More extensive glucuronidation in the female rat is unlikely as there was comparable overall recovery (urine + bile) of the rac-ketoprofen dose ($\approx$ 75%) as R- and S-

ketoprofen glucuronides in both female and male bile-duct cannulated rats. This would rule out the possibility of a gender difference in the total extent of glucuronidation of the enantiomers but not of relative gender differences in hepatic or extrahepatic tissue glucuronidation.

It was not possible to detect and quantify any ketoprofen glucuronides in the plasma of either male and female rats which is expected; assuming rapid clearance of the glucuronide by hydrolysis in plasma or by excretion into bile or urine. This was later supported by the experiments where R- or S-ketoprofen glucuronides were administered to rats and a rapid drop in plasma concentrations of the glucuronide following an *i.v.* bolus dose (Figure 24) coupled with a rise in concentration of deconjugated enantiomers were observed (Figure 25).

In addition to greater excretion of S-ketoprofen glucuronide into urine following formation, the higher urinary recovery of S-ketoprofen glucuronide and increased CL_{uconj} in female rats may also be explained by greater intrarenal glucuronidation of ketoprofen compared with male rats. Later experiments revealed significantly higher glucuronidation of ketoprofen enantiomers by female kidney microsomes when compared with microsomes prepared from male rats (Figure 12). This may be compensatory for lower hepatic activity in the female rat (Figure 10). There are numerous reports in the literature of renal glucuronidation being higher in female rats for a variety of aglycones (Graham and Skett, 1987; Mulder, 1986; Rush *et al.*, 1983).

The results of the pharmacokinetic studies in this work indicate that S-ketoprofen is very efficiently cleared from plasma in male rats by rapid excretion into bile as evidenced by the faster CL_{bconj}, resulting in more S-ketoprofen glucuronide being available for fecal elimination or deconjugation and recirculation in male rats than female rats. In female rats, the higher plasma concentrations and slower excretion of S-ketoprofen into bile suggests less efficient elimination *via* the biliary-fecal route. Rather, it appears S-ketoprofen is shunted to the kidney and excreted in urine. A compensatory relationship between urinary and biliary elimination is supported by the complementary S:R ketoprofen ratios of the glucuronides in bile (Male: 12.2 ± 2.4; Female: 6.8 ± 1.3) and urine (Male: 6.4 ± 4.2; Female: 13.7 ± 7.8). Compensation between urinary and biliary excretion has previously been described (Fleck and Braunlich, 1984) and the data in this work indicate that the relative contribution of each pathway may be gender dependent.

4.1.2 *In vitro* glucuronidation by rat microsomes

The actual mechanism(s) underlying the gender differences in biliary and renal clearance of S-ketoprofen remained unclear. As a result, the next step was to explore if the dimorphism in clearance pathways was secondary to sex dependent hepatic or renal glucuronidation in the rat which would influence the amount of glucuronide available for excretion and elimination

by a particular route.

A significant sex difference in the extent of hepatic (Figure 10) and renal (Figure 12) glucuronidation of rac-ketoprofen by rat microsomes was found. The male derived liver microsomes had higher activity than female while female derived kidney microsomes had higher activity than male. This is in agreement with reported sex differences for a variety of aglycones (Graham and Skett, 1987; Rush *et al.*, 1983; Chhabra and Fouts, 1974). Further to this, glucuronidation activity of both liver and kidney was manipulated by gonadectomy and/or hormonal replacement utilizing microsomes prepared from rats used in another experiment (see *2.3.2.2.*). There appeared to be a trend towards higher hepatic activity and lower renal glucuronidation in microsomes prepared from testosterone treated rats (Table VII).

The higher activity of microsomal enzymes from male rat liver has, in part, been attributed to an action of testosterone in increasing the magnitude of substrate binding capacity (Schenkman *et al.*, 1967). Androgens are known to be important regulators (Skett, 1988) and dominant factors in determining sex variations in drug metabolic enzymes and to influence glucuronidation *via* control of cofactor (UDPGA) availability in intact cells, tissue dependent UDPGT activity and active transport processes (Graham and Skett, 1987; Skett, 1987; Mulder, 1986). Others report that the effect of testosterone is closely related to its anabolic action and/or state of activation of UDPGT in different organs (Chhabra and Fouts, 1974).

If sex differences in metabolism arise as a result of the state of activation of UDPGT in different organs, then interpretation of data from *in vitro* microsomal tissue studies can be difficult. In most microsomal studies, as in this work, prior to incubation the microsomes are activated by a detergent or mechanical means to disrupt the membrane structure in order to liberate the membrane bound UDPGT. The observed activity then arises from the fully activated state of the enzyme and so is criticized as not being representative of the *in vivo* situation. This was the reason given in a study of sex differences in 1-naphthol glucuronidation in the rat where hepatic microsomal preparations revealed no sex differences in metabolism whereas a marked difference was seen utilizing liver cubes. A 3-fold higher activity was found in male rat liver cube preparations than those from female rat (Graham and Skett, 1987). In contrast, significant sex differences in hepatic and renal glucuronidation have been reported for 1-naphthol after maximal activation of microsomes by deoxycholic acid (Rush *et al.*, 1983) and for p-nitrophenol by the detergent Triton-X (Chhabra and Fouts, 1974). The methods used in this work were similar to those of Chhabra and Fouts (1974) employing the detergent Triton-X for activation and likewise, we were successful in detecting sex differences in glucuronidation.

In contrast to male rats, it is reported that renal microsomes from female rats may have higher glucuronidation activity (Rush *et al.*, 1983; Chhabra and Fouts, 1974). Correlations between renal excretion of

glucuronide metabolite(s) and renal microsomal UDPGT activity have been described for various substrates. Female rats excrete significantly more p-nitrophenol glucuronide in urine than males and have higher renal microsomal UDPGT activity toward p-nitrophenol than male rats although males have higher hepatic microsomal activity (Rush *et al.* 1983). Similar results have been found with 1-naphthol; however, in contrast, renal microsomes of either male or female rats failed to glucuronidate morphine and likewise no sex difference in urinary morphine glucuronide excretion was observed (Rush *et al.*, 1983).

The dependency of ketoprofen glucuronidation on the sex hormones was investigated by preparing liver and kidney microsomes from the rats used in the gonadectomy and sex hormone study. In doing so, an attempt to establish a similar correlation as per the work of Rush *et al.* (1983) between renal glucuronidation and urinary recovery of S-ketoprofen glucuronide was made. The result was a significant positive correlation ($r = 0.597$) between renal glucuronidation of S-ketoprofen and 24 h urinary recovery of S-ketoprofen glucuronide (Figure 13). The correlation may have been strengthened by a modification of the experimental technique as renal microsomes were prepared from whole kidney whereas Rush *et al.*, 1983 used only renal cortex which may have a bearing on enzymatic activity. Nonetheless, the existence of gender dependent intrarenal glucuronidation of ketoprofen in the rat was demonstrated. A strong negative correlation ($r = -$

0.859) between hepatic glucuronidation of S-ketoprofen and 24 h urinary recovery of S-ketoprofen glucuronide was found (Figure 11), thus, perhaps further supporting existence of a compensatory relationship for biliary and renal elimination of ketoprofen in the rat.

4.1.3. Gonadectomy and sex hormones

The pharmacokinetic and microsomal experiments suggested that the dimorphism in metabolism and disposition of S-ketoprofen in the rat were under control of the sex hormones. As a result, an attempt to determine if testosterone and estradiol are the hormones involved was made by investigating the effect of gonadectomy and these hormones on the urinary excretion of S-ketoprofen glucuronide, the pharmacokinetic parameter displaying the most marked gender dimorphism in the rats.

The 24 h urinary recovery of S-ketoprofen glucuronide remained virtually unchanged in ovarectomized female rats but rapidly increased in castrated male rats shortly after gonadectomy (Table VIII). This implies that testosterone may play a role in the regulation of hepatic glucuronidation of S-ketoprofen or sinusoidal translocation and/or excretion of the glucuronide into bile. Alternatively, testosterone may also be responsible for inhibiting renal excretion of S-ketoprofen glucuronide in the male rat. Tanaka *et al.*, 1991 reported such an action of testosterone as their findings revealed treatment of gonadectomized male and female rats with testosterone

resulted in suppression of urinary excretion of the aldose reductase inhibitor, zenarestat, characteristic of normal adult male rats. Similar to the observation with S-ketoprofen, castration alone resulted in a female pattern of urinary excretion of zenarestat in male rats whereas ovarectomy alone had little effect. This inhibitory action has also been demonstrated by testosterone's protective role in preventing nephrotoxicity in the rat. Male rats or testosterone treated ovarectomized female rats were protected from renal toxicity caused by administration of 2-aminoanthraquinone while normal females developed nephrotoxicity (Gothoskar and Weisburger, 1980).

The finding that testosterone treated ovarectomized female rats developed a male pattern of S-ketoprofen urinary excretion over time (Table VIII) further indicates that testosterone may play a role in inhibiting renal excretion of S-ketoprofen glucuronide. The apparent time lag (3 weeks) for urinary recovery of S-ketoprofen glucuronide to become significantly reduced within the ovarectomized + testosterone treated female rats may reflect the time required for testosterone to reach some threshold concentration that produces masculinization or perhaps for levels of estrogen to drop below that antagonizing testosterone (Figure 14). Further to this, the steady increase in urinary recovery of S-ketoprofen glucuronide with time in castrated + estradiol treated male rats (Figure 15) or decrease in ovarectomized + testosterone treated female rats (Figure 14) suggests that the androgen:estrogen ratio may be a critical factor in expression of sexual

dimorphism (Skett, 1987).

An even more pronounced and rapid increase in urinary recovery of S-ketoprofen glucuronide was observed in the castrated + estradiol treated male rats when compared with male rats that underwent castration alone (Figure 15). An action of estradiol, however, in promoting renal excretion is unlikely as in ovarectomized + estradiol treated female rats, there was no significant increase in urinary recovery of the glucuronide from that recovered from control or sham-operated females rats.

An interesting phenomenon in castrated + estradiol treated male rats was manifested as a failure to gain weight at a rate in accordance with other males (Figure 16). This may be an indirect reflection of the anabolic action of testosterone, as liver and kidney weight have been found to correlate with levels of testosterone (Blantz *et al.*, 1988; Gothoskar and Weisburger, 1980). In contrast, the other rats continued to gain weight at a rate similar to controls. It may be possible that the androgen:estrogen ratio is critical to overall control of anabolic activity and subsequent body weight.

Following the initial experiments, urinary excretion of S-ketoprofen glucuronide was investigated in sham-operated female and male rats to ensure that any changes were not an artifact of surgery. No apparent differences were noted in urinary recovery of S-ketoprofen glucuronide from that of control rats, thus, it can be assumed that surgery or anesthetic had no effect. In addition, the ability to maintain the female or male urinary

excretion pattern of S-ketoprofen glucuronide following surgery and hormonal replacement was investigated in ovarectomized + estradiol female rats and castrated + testosterone male rats. This was done to ensure changes in renal excretion were due to the presence and/or absence of estradiol and testosterone and not other sex hormones. In both cases, the excretion of S-ketoprofen glucuronide was not changed from control or sham-operated rats.

The data indicate no significant gender differences in the amount of R-ketoprofen glucuronide recovered in urine in control rats or following any hormonal perturbation. This may result from the major route of elimination of R-ketoprofen being extensive unidirectional bioinversion to the active S-antipode (Foster and Jamali, 1988). It can be assumed that if any sex difference in inversion in the rat exists, it is negligible and that gender differences in the pharmacokinetic parameters of S-ketoprofen result from a dimorphism in clearance pathways other than inversion.

4.1.4. Plasma protein binding

There was no significant difference in the extent of *in vitro* protein binding of R- or S-ketoprofen between male and female rat plasma, nor was any stereoselectivity in binding evident (Figure 17). As a result, the findings agree with those of Hayball *et al.*,1991, who used similar enantiomeric concentrations and ultrafiltration methodology to study the *in vitro* binding

of ketoprofen in human plasma and likewise found no stereoselectivity. There was a significant increase in the percent unbound of R-ketoprofen at enantiomeric concentrations of 50 μg/mL in male plasma and both R- and S-ketoprofen in male and female plasma at 100 μg/mL; however, both concentrations far exceed the normal therapeutic range (Figure 17). There are contradictory reports of stereoselective binding of ketoprofen; however, these studies have employed concentrations far exceeding therapeutic levels in addition to using solutions of lyophilised human serum albumin to simulate plasma (Dubois *et al.*, 1993a; Rendic *et al.*, 1980).

In light of albumin concentrations of the male and female plasma being identical and lack of stereoselectivity or gender differences in binding of the enantiomers, it can be assumed that plasma protein binding does not contribute to the observed gender difference in ketoprofen pharmacokinetics in the rat.

4.1.5. Intestinal permeability

It has been speculated that species are most sensitive to developing gastrointestinal lesions if biliary excretion of a drug exceeds renal excretion (Rollins and Klaassen, 1979). Furthermore, it has been shown that biliary excretion of intact and glucuronidated indomethacin is a primary causative factor of gastrointestinal lesions in the rat (Duggan *et al.*, 1975). As a result, it is plausible that gastrointestinal toxicity of NSAIDs differs between

females and males and may be determined, in part, by the relative contribution of biliary excretion and subsequent enterohepatic recirculation.

Gastrointestinal damage as measured by urinary recovery of ^{51}Cr-EDTA from male and female rats in our study did not reveal any gender differences in increased permeability following an acute oral dose of rac-ketoprofen. Despite the observed gender differences in CL_{bconj} of the enantiomers, the cumulative recovery of both R- and S-ketoprofen glucuronides remained similar in both sexes which may explain the lack of gender differences in intestinal permeability. The failure to see a difference in this study may also be due to the dose of ketoprofen being too small to detect slight gender differences in increased permeability. Another factor may be that gastrointestinal toxicity of ketoprofen may be more evident after chronic exposure rather than after an acute dose such as was used in this work.

There is also unresolved debate in the literature as to whether the actions of the NSAIDs on the gastrointestinal tract are mediated locally or systemically, thus, primarily if the gastrointestinal toxicity of ketoprofen is mediated systemically, one would not expect differences in biliary excretion to play a large role in overall increased permeability measured by ^{51}Cr-EDTA.

Overall the gender dimorphism in the stereoselective pharmacokinetics of ketoprofen in rats appears to be mediated through sex dependent liver and kidney glucuronidation coupled with or leading to a subsequent dimorphism

in renal and biliary excretion pathways. The results of these findings illustrate the importance of considering gender as an important variable in both human and animal pharmacokinetic studies.

4.2. Concentration dependent interaction of probenecid and ketoprofen in rats

4.2.1. Stereospecific assay

Initially an attempt was made to use a previously reported stereospecific HPLC assay for ketoprofen (Foster and Jamali, 1987) to quantitate probenecid and ketoprofen simultaneously. The assay was found unsuitable due to interference of the fenoprofen (internal standard) peaks with the probenecid peak, thus necessitating the use of a different internal standard. In addition, poor linear correlations between concentrations and peak area ratios were observed utilizing the previously reported extraction solvent volumes of 3 mL. In the previous method, linearity was investigated in the concentration range 0.05-5 μg/mL and the volume of 3 mL found to be adequate. In attempting to quantify ketoprofen and probenecid simultaneously in the range of 1-40 μg/mL, an inconsistency characterized by an upturn in the peak area ratio - concentration curve resulting in higher than expected ratios for concentrations greater than 20 μg/mL was observed. It was found that increasing the extraction volumes to 5 mL resulted in excellent linearity ($r^2 > 0.999$) and consistent peak area ratios

(Table IV).

The modification of the ketoprofen assay resulted in a method that is rapid, stereospecific and convenient and is applicable to pharmacokinetic studies that require simultaneous determination of probenecid, ketoprofen enantiomers and their glucuronides in plasma and urine. The simultaneous quantitation of probenecid coadministered with other drugs in biological samples is imperative in order to ascertain if the interaction is a competitive and dose dependent process.

4.2.2. Disposition in rats

Interactions involving probenecid and NSAIDs are extensive and include naproxen (Runkel *et al.*, 1978), indomethacin (Baber *et al,*, 1978), diflusinal (McKinnon and Dickinson, 1989), zomepirac (Smith *et al.*, 1985), carprofen (Spahn *et al.*, 1989), benoxaprofen (Spahn *et al.*, 1987) and ketoprofen (Upton *et al.*, 1982). Previous studies report that the interaction between ketoprofen (Upton *et al.*, 1982) or ketoprofen enantiomers (Foster *et al.*, 1989) with probenecid results from competition for glucuronide conjugation and subsequent excretory pathways.

Complications may arise when studying drug interactions with probenecid if the extent of interaction and resulting pharmacokinetic and pharmacodynamic outcomes depend upon the amount of probenecid present. If an administered probenecid dose results in levels below the

minimum required to elicit an interaction, it may be erroneously concluded that no interaction occurs at any dose level. Owing to the competitive nature of the probenecid - ketoprofen interaction, an inhibition will occur only as long as probenecid is present in the system. This was evidenced by the observation of probenecid being rapidly eliminated with levels no longer detectable in male or female rat plasma after the 8-12 h collection time following a 100 mg/kg *i.v.* dose (Figures 19 & 23). In this work, a single *i.v.* dose of probenecid was used prior to rac-ketoprofen to investigate the dependency of the ketoprofen enantiomer concentrations on various probenecid doses in the female rat (Table IX). A different outcome may result from the administration of smaller repeated doses or a constant infusion of probenecid where steady state plasma concentration should be that required to competitively inhibit elimination of the coadministered drug. In light of this consideration, interactions may be observed with substantially lower doses in male and female rats than were observed in our experiments.

Both ketoprofen enantiomers were affected by the presence of probenecid in female and male rats (Tables IX and XIV). It was evident that the pattern of elevated AUC was not as pronounced for R-ketoprofen as was observed for S-ketoprofen in either sex. This suggests some degree of stereoselectivity in the effect of probenecid on ketoprofen pharmacokinetics.

The progressive increase in the urinary S:R ketoprofen glucuronide ratios observed in the female rats with increasing probenecid dose (Table XII) was

thought to perhaps indicate an enhanced extent of chiral inversion. This could be attributed to inhibited elimination of R-ketoprofen resulting in its being made more available to the site(s) of metabolic inversion. A similar increase in inversion for the enantiomers of 2-phenylpropionic acid has been reported in rabbits with experimental renal failure (Meffin *et al.*, 1986). An increased inversion alone; however, cannot completely account for such a drastic rise in S:R ketoprofen ratios. If one assumes approximately 80% inversion in the absence of probenecid such as previously reported for male Sprague Dawley rats (Iwakawa *et al.*, 1991; Foster and Jamali, 1988) it is unlikely that a further 20% increase in inversion can account for the observed difference. Furthermore, inversion was not observed in any of the later *in vitro* microsomal studies of ketoprofen in the presence of probenecid (Figures 27 & 28). It is more reasonable to attribute this rise in S:R ratios to altered disposition of the enantiomers in the presence of probenecid.

The CL_{uconj} values for both R- and S-ketoprofen were found to decrease with an increase in probenecid dose in both female and male rats (Tables XI and XIV). This is consistent with glucuronidation and subsequent renal elimination of S-ketoprofen being inhibited by probenecid as one would expect slower renal clearance of S-ketoprofen glucuronide conjugate in the presence of probenecid. However, it is of interest that the data reveals no marked reduction in the $\Sigma Xu_{urine(0\text{-}24\ h)}$ of S-ketoprofen compared to controls. This may be due to the fact that only a single dose of probenecid was given

in these studies. Once probenecid levels fall below those required to elicit an interaction, competition is removed and, subsequently the percentage of glucuronidated ketoprofen excreted within a 24 h period may remain very similar as eventually all the drug will be eliminated.

A marked dependency of R- and S-ketoprofen plasma concentrations and AUC on those of probenecid was found in female rats (Figures 21 and 22). Concentration and/or resultant AUC of probenecid as opposed to dose was found to be a better indicator of the extent of interaction. Initially, attempts to establish a positive relationship between $AUC_{(0\text{-}24h)}$ of R-ketoprofen ($r = 0.221$) and S-ketoprofen ($r = 0.465$) with probenecid dose met with little success. The poor correlations can be attributed to the nonlinear pharmacokinetics of probenecid previously reported for doses higher than 50 mg/kg in the rat (Emanuelson and Paalzow, 1986) and are further supported by the nonlinear relationship observed between the probenecid doses used in this study and corresponding AUCs (Figure 20).

The progressive rise in S:R-ketoprofen ratios in female rats correlated well with increasing probenecid dose (mg/kg) as opposed to AUC (Table XII). The better correlation with dose may be attributed to the nonlinear kinetics of probenecid which resulted in increasing $t_{1/2}$ with increasing probenecid dose. Due to the error involved in determining $t_{1/2}$ at each dose of probenecid, we chose instead to relate S:R ratios to probenecid MRT. MRT was calculated for the various concentrations of probenecid indicated in

Table IX and was used only for qualtitative comparison and correlation with the S:R ratios. The results indicate similar significant positive correlations between S:R ratios and probenecid MRT as were seen with $t_{1/2}$.

An attempt was made to measure R- and S-ketoprofen glucuronide conjugates in male and female rat plasma as previous works have reported elevated levels of glucuronides in human plasma for carprofen (Spahn *et al.*, 1989) and benoxaprofen (Spahn *et al.*, 1987) and for ketoprofen (Upton *et al.*, 1982) measured nonstereospecifically when coadministered with probenecid. No appreciable amounts of glucuronidated R- or S-ketoprofen in rat plasma could be found. Furthermore, the subsequent *in vivo* experiments with R- and S-ketoprofen glucuronides indicate rapid clearance of the conjugates even in the presence of probenecid (Figure 24).

A larger extent of interaction of S-ketoprofen (measured by increase in AUC) with probenecid in female rats was seen when compared with male rats (Tables IX & XIV). A plausible explanantion may be that quantitatively probenecid cannot compete for biliary excretion with glucuronidated S-ketoprofen in male rats to the extent that it can in female rats due to the efficiency of biliary-fecal clearance in the male rat as discussed under *4.1.1.* The small contribution of the renal pathway to overall elimination of S-ketoprofen in the male rat coupled with insignificant changes in CL_{uconj} suggests that the increased S-ketoprofen AUC in male rats in the presence of probenecid results primarily from competition for CL_{bconj}.

Probenecid can also compete for glucuronide formation (Sorgel *et al.*, 1980) prior to biliary or renal excretion of formed conjugates or for reconjugation of recirculated drug. It follows that, if glucuronidation activity of male rat liver is greater than female rat liver, probenecid may exhibit quantitatively less competition at this level in the male than female rat. An attempt to investigate this was made in the experiments where the *in vitro* glucuronidation of ketoprofen enantiomers in the presence of probenecid (Figures 27& 28) was studied; however, no gender differences in the extent of inhibition of glucuronidation by probenecid were found.

In spite of the marked gender difference in the amount of S-ketoprofen glucuronide excreted in urine, probenecid caused similar decreases in CL_{uconj} in both female (55%) and male (49%) rats. This may further support the assumption that gender differences in the interaction of S-ketoprofen with probenecid are primarily manifested at the level of biliary rather than renal clearance.

The interaction between ketoprofen and probenecid was found to be concentration dependent, stereoselective and to have a gender component in the rat. These findings serve to illustrate the complexity and many facets that must be considered in elucidation of the mechanism of a drug interaction.

4.2.3. *In vivo* disposition of ketoprofen glucuronides

To delineate further the mechanism involved in the probenecid - ketoprofen interaction, an attempt to administer glucuronidated ketoprofen enantiomers concomitantly with probenecid was made. By giving the formed glucuronides it was hoped that the role of probenecid in competing for excretory route(s) with enantiomer glucuronides apart from probenecid's action in competing for formation of the glucuronides could be characterized. The dose of 100 mg/kg of probenecid was used in order to be consistent with the earlier work (*see 2.3.2.4.1. & 2.*).

There are numerous difficulties in attempting to obtain, administer and study disposition of glucuronide conjugates as they are inherently unstable and pose analytical difficulties (Smith *et al.*, 1985). Since we were unable to obtain ketoprofen enantiomer glucuronides from any commercial source, they were obtained following collection of urine after an oral dose of ketoprofen in a human volunteer. There was great difficulty in obtaining a high enough concentration in the urine so as to avoid having to inject a large volume of the filtered urine into the rat, hence relatively low doses ($\approx$ 1 mg/kg) of the glucuronides were given. Nonetheless, even at these low doses, a competitive role of probenecid in reducing elimination of ketoprofen glucuronides in rats irrespective of competition for glucuronidation was observed.

When ketoprofen enantiomer glucuronides were given alone, a rapid drop

in the mean plasma concentrations of both enantiomer glucuronides were observed in the first hour which may be attributed to rapid clearance through hydrolysis to parent drug in plasma or by rapid biliary or renal excretion of the polar glucuronides (*see insert,* Figure 24). Rapid *in vivo* hydrolysis of S-glucuronide to its respective aglycone is supported by the concomitant rise in the first hour in the mean plasma concentration of deconjugated S-ketoprofen (*see insert,* Figure 25). The plasma concentration of deconjugated R-ketoprofen does not rise in a similar fashion, perhaps due to competitive clearance by its rapid bioinversion to S-ketoprofen in the rat.

Rapid biliary excretion was evidenced by the amount of glucuronides collected in bile from 0-3 h in bile-duct cannulated rats (Figure 26). Furthermore, the significant contribution to AUC of enterohepatic recirculation of the glucuronides is supported by the very small plasma AUC of both ketoprofen enantiomer glucuronides in bile-duct cannulated rats when compared with intact rats. Rapid urinary excretion of the glucuronides was evident by the recovery of a large percentage of the total amount of glucuronides excreted in urine within 0-3 h in intact rats (*see 3.3.3.1.*).

When probenecid was given with the ketoprofen enantiomer glucuronides, a significant increase in plasma AUC and decrease in CL_{TB} of S-ketoprofen glucuronide was seen (Figure 24). This supports a competitive role of probenecid or its glucuronide competing for elimination with the formed S-ketoprofen glucuronide. A trend towards a similar increase in R-

ketoprofen glucuronide AUC did not reach statisitical significance. Both AUC of deconjugated R- and S-ketoprofen were significantly higher when probenecid was given concomitantly, owing perhaps to accumulation of the glucuronide in plasma resulting in extensive hydrolysis or a competitive inhibition of reconjugation of deconjugated enantiomers by probenecid.

There was significantly less (≈ 50%) cumulative biliary recovery of both R- and S-ketoprofen glucuronides in the first 3 h following the dose of glucuronides in bile-duct cannulated rats in the presence of probenecid (Figure 26). It seems likely that the major role of probenecid in its interaction with ketoprofen is manifested at the level of biliary rather than renal clearance as there was not a significant reduction in the amount of glucuronides recovered in either the first 3 hours or in the 24 h urine collection following the dose of glucuronides when given with probenecid in intact rats (*see 3.3.3.1.)*.

The failure to see a substantial decrease in urinary recovery of ketoprofen glucuronides in the presence of probenecid was observed in the previous experiments in both female and male rats (Tables IX & XIV). Furthermore, the observed gender difference in the extent of interaction of probenecid with ketoprofen in the rat was explained by probenecid's role in competing for biliary elimination rather than renal elimination (*see 4.2.2.*). The data from this experiment suggest that a major action of probenecid or its glucuronide is to competitively inhibit some aspect of ketoprofen glucuronide

excretion into bile either at the level of entry into the hepatocyte or for biliary transport. This does not preclude a role of probenecid in competing with ketoprofen for glucuronidation, thereby influencing the amount of glucuronide available for excretion into bile which will be discussed next.

4.2.4. *In vitro* glucuronidation by rat microsomes

The ability of probenecid to compete for glucuronidation of ketoprofen was investigated by utilizing microsomal preparations of rat liver and kidney tissues. A common practice in interaction studies with probenecid is to administer high doses of probenecid such that its plasma concentration greatly exceeds that of the coadministered drug. Since the degree of interaction may depend upon the amount of probenecid that is present as shown in the concentration dependency work (Table IX), it was decided to investigate the effect of three concentrations of probenecid on the net glucuronidation of ketoprofen enantiomers. These concentrations were representative of the situations where probenecid molar concentrations were in great excess, equivalent to and lower than those of rac-ketoprofen (Figure 27 & 28).

The data indicate that as probenecid concentration is increased, the amount of R-ketoprofen or S-ketoprofen glucuronide formed by liver microsomes is decreased (Figure 27). A similar trend of probenecid concentration dependent inhibition of glucuronidation seems likely in renal

microsomes; however, owing to large variability and the small amount of glucuronide formed in this tissue, the data failed to reach statistical significance (Figure 28).

Overall, the data indicate that the probenecid - ketoprofen interaction is concentration dependent, stereoselective and displays a gender dimorphism in the rat. The interaction appears to result from a concentration dependent role of probenecid in competing for formation of ketoprofen enantiomer glucuronides coupled with an action in inhibiting elimination of formed glucuronides primarily *via* biliary rather than renal clearance.

5. CONCLUSIONS

The marked gender dimorphism in the stereoselective pharmacokinetics of ketoprofen in rats can be attributed to sex hormone dependent hepatic and renal glucuronidation coupled with or leading to sex differences in renal and biliary clearance of formed enantiomer glucuronides. No gender difference or stereoselectivity in the plasma protein binding of ketoprofen in rat plasma at normal therapeutic concentrations was found. A summary of the major pharmacokinetic differences of the active S-enantiomer of ketoprofen between male and female rats is given in Table XV. In both sexes, the S-ketoprofen enantiomer predominates in plasma as there is rapid clearance of R-ketoprofen through bioinversion to the S-antipode which is known to occur systemically and in the gut in the rat. As is shown in Figure 29, the fate of S-ketoprofen or the small amount of R-ketoprofen present in plasma involves hepatic glucuronidation to the corresponding glucuronide conjugate with subsequent biliary excretion of the conjugate and elimination in feces or excretion into the intestine whereupon it may be deconjugated and recirculated to plasma. In addition, the enantiomer conjugates may be eliminated directly in urine or the enantiomers themselves may undergo renal glucuronidation and subsequent renal excretion of the formed conjugate. Due to the number of processes involved in the stereoselective disposition of ketoprofen in the rat, elucidation of the actual mechanism(s) under hormonal control is difficult and complicated by the fact they occur simultaneously.

Table XV. Summary of S-ketoprofen pharmacokinetic parameter differences between female and male rats[1]

Pharmacokinetic parameter	Gender difference
$AUC_{(0\text{-}24\ h)}$	♀ >> ♂
CL_{TB} (mL/min)	♀ << ♂
ΣXu_{urine} of glucuronide	♀ >>> ♂
ΣXu_{bile} of glucuronide	♀ = ♂
CL_{bconj}	♀ < ♂
Hepatic glucuronidation	♀ << ♂
Renal glucuronidation	♀ >> ♂
Unbound fraction in plasma	♀ = ♂
Extent of interaction with probenecid	♀ >> ♂

[1] ♀, female rats; ♂, male rats

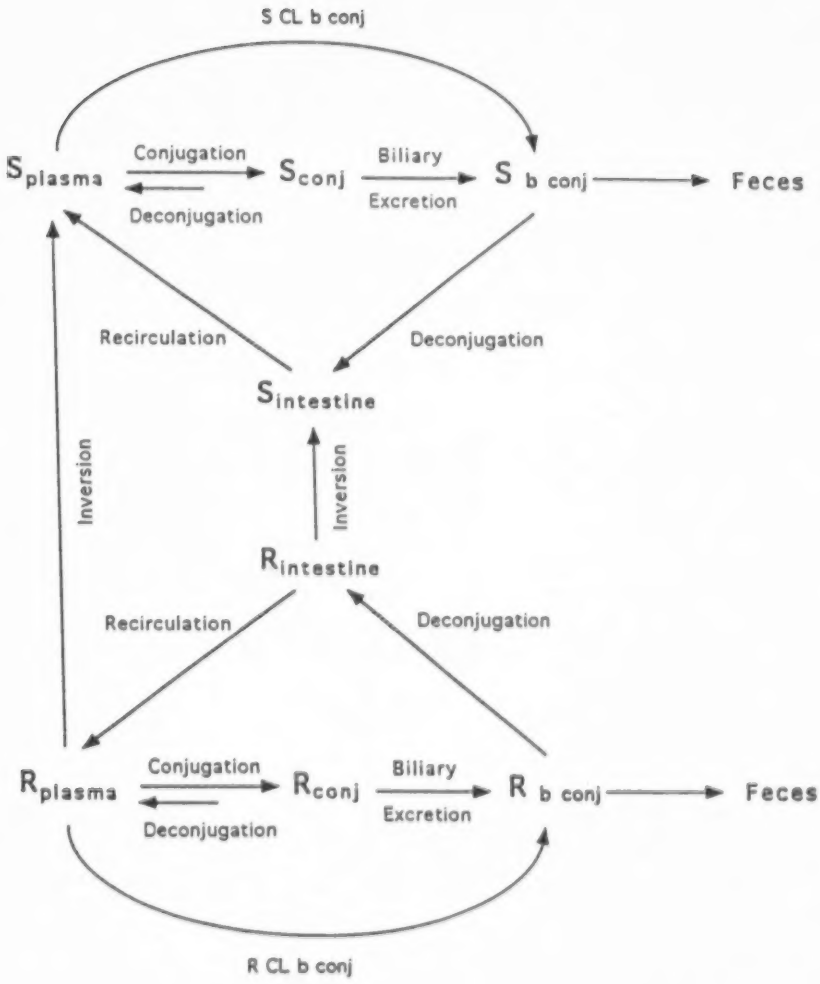

Figure 29. Disposition of R-ketoprofen and S-ketoprofen following *i.v.* administration of rac-ketoprofen in the rat. The following symbols represent: S_{plasma}, R_{plasma} = enantiomer in plasma; S_{conj}, R_{conj} = enantiomer glucuronide conjugate; S_{bconj}, R_{bconj} = enantiomer glucuronide conjugate eliminated *via* biliary excretion; $S_{intestine}$, $R_{intestine}$ = deconjugated enantiomer in intestine

The data offer a plausible explanation for the gender dimorphism in ketoprofen pharmacokinetics in the rat. It appears in male rats, that due to greater hepatic glucuronidation of S-ketoprofen, there in turn is more glucuronide available for clearance *via* biliary excretion and fecal elimination than in the female rat. Indeed, it was found that CL_{bconj} of S-ketoprofen was significantly greater in the male rat than in the female rat. The lower hepatic glucuronidation of S-ketoprofen in the female rat results in less glucuronide being available and so less efficient excretion of S-ketoprofen glucuronide into bile and clearance *via* this route in female rats. The high plasma levels of S-ketoprofen in the female rat expose the kidney to an appreciable concentration of drug. Due to higher glucuronidation activity of female rat kidney, it appears that intrarenal glucuronidation occurs leading to enhanced excretion of S-ketoprofen glucuronide into urine in the female rat. Furthermore, a positive correlation of renal glucuronidation and a strong negative correlation of hepatic glucuronidation of S-ketoprofen with urinary recovery of S-ketoprofen glucuronide was found. Sex dependent active transport processes regulating transport of the glucuronides or sex differences in deconjugating activity of formed glucuronides *in vivo* cannot be ruled out, however, the latter is probably unlikely. Due to the lability of acyl-glucuronides in rat plasma, one would not expect marked gender differences in deconjugation and in fact no ketoprofen enantiomer glucuronides could be detected in plasma of either male or female rats. The

observation of rapid clearance of ketoprofen enantiomer glucuronides after administration of the glucuronides supports this notion.

In both female and male rats, renal excretion of S-ketoprofen glucuronide and hepatic or renal glucuronidation activity could be altered by gonadectomy with or without concurrent estradiol or testosterone treatment. This demonstrates a marked dependence of the dimorphism in pharmacokinetics on these sex hormones, although the exact mechanism by which the hormones exert their action in the rat remains unknown.

There were no gender differences in the increased urinary excretion of ^{51}Cr-EDTA as a marker of intestinal permeability changes following an oral dose of rac-ketoprofen. The failure to see a difference may have resulted from the dose of ketoprofen being too small to detect slight gender differences in increased permeability or perhaps the gastrointestinal toxicity of ketoprofen may be more evident after chronic exposure rather than after an acute dose such as was studied in this work. Nonetheless, it is important to emphasize that studies of NSAIDs performed solely in male rats may not reflect the species as a whole especially since the female may exhibit contrasting physiologic responses with regard to the prostaglandin system.

The interaction of probenecid and ketoprofen was studied in female and male rats and was found to be stereoselective and probenecid concentration dependent. There was a gender difference in the extent of interaction of probenecid with S-ketoprofen attributable to the underlying gender

dimorphism in the pharmacokinetics of ketoprofen, although qualitatively the interaction is similar in both sexes. Probenecid coadministration resulted in a significantly lower recovery of S-ketoprofen glucuronide in bile and decreased hepatic glucuronidation of both ketoprofen enantiomers. As a result, if coadministration of probenecid results in reduced biliary elimination of S-ketoprofen coupled with inhibition of glucuronidation or reconjugation of recirculated S-ketoprofen that has entered bile, the result will be prolonged and elevated levels of the S-antipode which may have therapeutic consequences.

Gender differences in the human population of NSAID users have not been well addressed to date. It is realized that substantial interspecies differences exist in the pharmacokinetics of ketoprofen and it follows that the influence of the sex hormones on ketoprofen disposition may be entirely different between rats and humans. Nonetheless, in the future an animal model reflecting a marked dimorphism in the disposition of these agents, such as we have described, may be invaluable in order to study the mechanisms involved and resulting hormonal dependencies in the human population. Due to the slower CL_{biconj} and greater cumulative urinary excretion of S-ketoprofen glucuronide, the female rat may be a more representative animal model for pharmacokinetic and drug interaction studies than the male rat as the major route of elimination of glucuronidated ketoprofen enantiomers in the human is *via* the renal route.

This research project highlights the importance of considering gender in pharmacokinetic investigations. Although the dramatic differences seen between male and female rats are not expected in humans, the fact that the urinary excretion pattern of S-ketoprofen in the female rat resembles the human pattern more closely than the male is an important finding. Indeed, studies on the influence of pathophysiological changes may be more relevant if a more appropriate animal model is used. Our investigation into the probenecid - ketoprofen interaction highlights this point as the extent of interaction was greater in the female rat.

Gender is an important research variable that can obscure or clarify scientific results. It is unfortunate that many researchers will control for this variable by using only male subjects in studies addressing therapeutic questions affecting the entire population. It is true that the numbers of clinically significant gender differences in the pharmacokinetics or pharmacodynamics of a drug that neccessitate dosage regimen modification are small, however, even these numbers would not be realized had gender not been considered.

6. REFERENCES

Aarons, L., Hopkins, K., Rowland, M., Brossel, S. and Thiercelin, J.F.: *Route of administration and sex differences in the pharmacokinetics of aspirin, administered as its lysine salt.* Pharm. Res. 6:660-666, 1989.

Abas, A. and Meffin, P.J.: *Enantioselective disposition of 2-arylpropionic acid nonsteroidal antiinflammatory drugs IV. Ketoprofen disposition.* J. Pharmacol. Exp. Ther. 240:637-641, 1987.

Aldercreutz, H. and Tenhunen, R.: *Some aspects of the interaction between natural and synthetic female hormones and the liver.* Am. J. Med. 49:630-648, 1970.

Ariens, E.J.: *Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology.* Eur. J. Clin. Pharmacol. 26:663-668, 1984.

Avouac, B. and Teule, M.: *Ketoprofen: The European experience.* J. Clin. Pharmacol. 28:S2-S7, 1988.

Baber, N., Halliday, L., Sibeon, R., Littler, T. and Orme, M.L'E.: *The interaction between indomethacin and probenecid.* Clin. Pharmacol. Ther. 24:298-307, 1978.

Banwarth, B., Lapicque, F., Netter, P., Monot, C., Tamisier, J.N.: *The effect of food on the systemic availability of ketoprofen.* Eur. J. Clin. Pharmacol. 33:643-645, 1988.

Bertagni, P., Hirom, P.C., Millburn, P., Osiyemi, F.O., Smith, R.L., Turbert, H.B. and Williams, R.T.: *Sex and species differences in the biliary excretion of tartrazine and lissamine fast yellow in the rat, guinea-pig and rabbit. The influence of sex hormones on tartrazine excretion in the rat.* J. Pharm. Pharmacol. 24:620-624, 1972.

Bjarnason, I., So, A., Levi, A.J., Peters, T.J., Williams, P., Zanelli, G.D., Gumpel, J.M. and Ansell, B.: *Intestinal permeability and inflammation in arthritis: Effects of nonsteroidal anti-inflammatory drugs.* Lancet. 8413:1171-1174, 1984.

Bjorkman, S.: *Determination of the enantiomers of ketoprofen in blood plasma by ion-pair extraction and high-performance liquid chromatography of leucinamide derivatives.* J. Chromatogr. 414:465-471, 1987.

Bjorkman, S.: *Determination of the enantiomers of indoprofen in blood-plasma by high-performance liquid chromatography after rapid derivatization by means of ethyl chlorformate.* J. Chromatogr. 339: 339-346, 1985.

Blantz, R.C., Peterson, O.W., Blantz, E.R. and Wilson, C.B.: *Sexual differences in glomerular ultrafiltration: Effect of androgen administration in ovarectomized rats.* Endocrinology. 22:767-773, 1988.

Blessington, B., Crabb, N., Karkee, S. and Northage, A.: *Chromatographic approaches to the quality control of chiral propionate antiinflammatory drugs and herbicides.* J. Chromatogr. 469:183-190, 1989.

Bonate, P.: *Gender-related differences in xenobiotic metabolism.* J. Clin. Pharmacol. 31:684-690, 1991.

Borsa, M., Tonon, G.C., Ronchi, C., Zanolo, G., Canali, S.: *Pharmacokinetics of a slow-release preparation of ketoprofen lysine in man.* Arzneim. -Forsch. / Drug Res. 33:1497-1500, 1983.

Bruguerolle, B. and Bouvenot, G.: *Lack of evidence of ketoprofen kinetic changes during the oestrous cycle in rats.* Fundem. Clin. Pharmacol. 5:347-350, 1991.

Brune, K., Geisslinger, G. and Menzel-Soglowek, S.: *Pure enantiomers of 2-arylpropionic acids: Tools in pain research and improved drugs in rheumatology.* J. Clin. Pharmacol. 32:944-952, 1992.

Buchanan, M.R., Rischke, J.A., Butt, R., Turpie, A.G.G., Hirsh, J. and Rosenfeld, J.: *The sex-related differences in aspirin pharmacokinetics in rabbits and man and its relationship to antiplatelet effects.* Thromb. Res. 29:125-139, 1983.

Caille, G., Du Souich, P., Gervais, P. and Besner, J.G.: *Single dose pharmacokinetics of ketoprofen, indomethacin and naproxen taken alone or with sucralfate.* Biopharm. Drug Disp. 8:173-183, 1987.

Caillleteau, J.G.: *Ketoprofen in dentistry: A pharmacologic review.* Oral Surg. Oral Med. Oral Pathol. 66:620-624, 1988.

Caldwell, J.: *The importance of stereochemistry in drug action and disposition.* J. Clin. Pharmacol. 32:925-929, 1992.

Caldwell, J., Hutt, A.J. and Fournel-Gigleux, S.: *The metabolic chiral inversion and disposition of the 2-arylpropionic acids and their biological consequences*. Biochem. Pharmacol. 37:105-124, 1988.

Campanella, C. and Jamali, F.: *Tenoxicam gender differences in the pharmacokinetics and pharmacodynamics in the rat.* J. Pharm. Sci. (submitted for publication), 1993.

Carraro-Eduardo, J.C., Oliveira, A.V., Carrapatoso, M.E. and Ornellas, J.F.R.: *Effect of sex hormones on gentamicin-induced nephrotoxicity in rats.* Brazilian J. Med. Biol. Res. 26:653-662, 1993.

Carrio, I., Notivol, R., Estorch, M., Berna, L. and Vilardell, F.: *Gender-related differences in gastric emptying.* J. Nucl. Med. 29:573-574, 1988.

Chhabra, R.S. and Fouts, J.R.: *Sex differences in the metabolism of xenobiotics by extrahepatic tissue in rats.* Drug Metabol. Disp. 2:375-379, 1974.

Collier,S. and Ghosh, P.: *Evaluation of the effects of antiarthritic drugs on the secretion of proteoglycans by lapine chondrocytes using a novel assay procedure.* Ann. Rheum. Dis. 48:372-381, 1989.

Compendium of Pharmaceuticals and Specialties: *Drug listing of brand and nonproprietary names.* ed. by C.M.E. *et al.*, pp. G18, Canadian Pharmaceutical Association, Ottawa, 1993.

Conway, W.D. and Melethil, S.: *Excretion of probenecid and its metabolites in bile and urine of rats.* J. Pharm. Sci. 63:1551-1558, 1974.

Corrigan, B. and Jamali, F.: *The effects of gender and sex hormones on the pharmacokinetics of etodolac in the rat.* Pharm. Res. Suppl. 10:S306, 1993.

Cunningham, R.F., Israili, Z.H. and Dayton, P.G.: *Clinical pharmacokinetics of probenecid.* Clin. Pharmacokinet. 6:135-151, 1981.

Datz, F.L., Christian, P.E. and Moore, J.: *Gender-related differences in gastric emptying.* J. Nucl. Med. 28:1204-1207, 1987.

Davison, J.S., Davison, M.C. and Hay, D.M.: *Gastic emptying in late pregnancy and labour.* J. Obstet. Gynaecol. Br. Commonwealth. 77:37-41, 1970.

Delbarre, F., Roucayrol, J.J., Amor, B., Ingrand, J. and Bourat, G.: *Pharmacokinetic study of ketoprofen (19.538 R.P.) in man using the tritiated compound.* Scand. J. Rheumatol. Suppl. 14:45-52, 1976.

Dubois, N, Lapicque, F., Abiteboul, M. and Netter, P.: *Stereoselective protein binding of ketoprofen: Effect of albumin concentration and of the biological system.* Chirality. 5:126-134, 1993a.

Dubois, N., Lapicque, F., Maurice, M.H., Pritchard, M., Fournel-Gigleux, S., Magdalou, J., Abiteboul, M., Siest, G. and Netter, P.: *In vitro irreversible binding of ketoprofen glucuronide to plasma proteins.* Drug Metabol. Disp. 21:617-623, 1993b.

Duggan, D.E., Hooke, K.F., White, S.D., Noll, R.M. and Stevenson, C.R. *The effects of probenecid upon the individual components of indomethacin elimination.* J. Pharmacol. Exp. Ther. 201:463-470, 1977.

Duggan, D.E., Hooke, K.F., Noll, R.M. and Kwan, K.C.: *Enterohepatic circulation of indomethacin and its role in intestinal irritation.* Biochem. Pharmacol. 25:1749-1754, 1975.

Ellison, K.E., Ingelfinger, J.R., Pivor, M. and Dzau, V.J.: *Androgen regulation of rat renal angiotensinogen messenger RNA expression.* J. Clin. Invest. 83:1941-1945, 1989.

Emanuelsson, B.M. and Paalzow, L.K.: *Hepatic and renal clearances of probenecid in the rat.* Pharmacol. 38:61-68, 1989.

Emanuelsson, B.M. and Paalzow, L.K.: *Dose-dependent pharmacokinetics of probenecid in the rat.* Biopharm. Drug Disp. 9:59-69, 1988.

Evans, A.M.: *Enantioselective pharmacodynamics and pharmacokinetics of chiral nonsteroidal antiinflammatory drugs.* Pharmacol. 42:237-256, 1992.

Evans, A.M., Nation, R.L., Sansom, L.N., Bochner, F. and Somogyi, A.A.: *Stereoselective drug dispositon: Potential for misinterpretation of drug disposition data.* Br. J. Clin. Pharmacol. 26:771-780, 1988.

Faed, E.: *Properties of acyl glucuronides: Implications for studies of the pharmacokinetics and metabolism of acidic drugs.* Drug Metabol. Rev. 15(5&6):1213-1249, 1984.

Fleck, C. and Braunlich, H.: *Methods in testing interrelationships between excretion of drugs via urine and bile.* Pharmacol. Ther. 25:1-22, 1984.

Flouvat, B., Sthenew, A. and Massias, P.: *Pharmacokinetic profile of a sustained-release preparation of ketoprofen.* Semaine des Hopitaux de Paris. 59:3187-3190, 1989.

Foster, R.T., Jamali, F. and Russell, A.S.: *Pharmacokinetics of ketoprofen enantiomers in cholecystectomy patients: influence of probenecid.* Eur. J. Clin. Pharmacol. 37:589-594, 1989a.

Foster, R.T., Jamali, F. and Russell, A.S.: *Ketoprofen enantiomers in synovial fluid.* J. Pharm. Sci. 78:881-882, 1989b.

Foster, R.T., Jamali, F., Russell, A.S. and Alballa, S.R.: *Pharmacokinetics of ketoprofen enantiomers in healthy subjects following single and multiple doses.* J. Pharm. Sci. 77:70-73, 1988a.

Foster, R.T., Jamali, F., Russell, A.S. and Alballa, S.R.: *Pharmacokinetics of ketoprofen enantiomers in young and elderly arthritic patients following single and multiple doses.* J. Pharm. Sci. 77:191-195, 1988b.

Foster, R.T. and Jamali, F.: *Stereoselective pharmacokinetics of ketoprofen in the rat: Influence of route of administration.* Drug Metabol. Disp. 16:623-626, 1988.

Foster, R.T. and Jamali, F.: *High performance liquid chromatographic assay of ketoprofen enantiomers in human plasma and urine.* J. Chromatogr. 416:388-393, 1987.

Fossgreen, J.: *Ketoprofen: A survey of current publications.* Scand. J. Rheumatol. Suppl. 14:7-32, 1976.

Frezza, M., di Padova, C., Pozzato, G., Terpin, M., Baraona, E. and Liber, C.S.: *High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism.* New Engl. J. Med. 322:95-99, 1990.

Gibaldi, M. and Perrier, D. *Pharmacokinetics.* Marcel Dekker, New York, 1982.

Gilmore, D.A., Gal, J., Gerber, J.G. and Nies, A.S.: *Age and gender influence the stereoselective pharmacokinetics of propranolol.* J. Pharmacol. Exp. Ther. 261:1181-1186, 1992.

Giudicelli, J.F. and Tillement, J.P.: *Influence of sex on drug kinetics in man.* Clin. Pharmacokinet. 2:157-166, 1977.

Gothoskar, S.V. and Weisburger, E.K.: *Protection by testosterone propionate against the nephrotoxicity of 2-aminoanthraquinone in Fischer rats.* Med. Biol. 58:281-284, 1980.

Graham, D.L. and Skett, P.: *Endocrine regulation of the metabolism of 1-napthol in the rat.* J. Pharmacol. Exp. Ther. 242:344-348, 1987.

Grossman, M.I., Kirsner, J.B. and Gillespie, I.A.: *Basal and histalog-stimulated gastric secretion in control subjects and in patients with peptic ulcer or gastric cancer.* Gastroenterol. 45:14-26, 1963.

Gustafsson, J.A., Eneroth, P., Pousette, A., Skett, P., Sonnenschein, C., Stenberg, A. and Ahlen, A.: *Programming and differentiation of rat liver enzymes.* J. Steroid Biochem. 8:429-443, 1977.

Gustafsson, J.A. and Stenberg, A.: *On the obligatory role of the hypophysis in sexual differentiation of hepatic metabolism in rats.* Proc. Natl. Acad. Sci. USA. 73:1462-1465, 1976.

Hashimoto, S., Miwa, M., Akasofu, K. and Nishida, E.: *Changes in 40 serum proteins of post-menopausal women.* Maturitas. 13:23-33, 1991.

Hayball, P.J., Nation, R.L., Bochner, F., Sansom, L.N., Ahern, M.J. and Smith, M.D.: *The influence of renal function on the enantioselective pharmacokinetics and pharmacodynamics of ketoprofen in patients with rheumatoid arthritis.* Br. J. Clin. Pharmacol. 36:185-193, 1993.

Hayball, P.J., Nation, R.L. and Bochner, F.: *Steroselective interactions of ketoprofen glucuronides with human plasma protein and serum albumin.* Biochem. Pharmacol. 44:291-299, 1992a.

Hayball, P.J., Nation, R.L. and Bochner, F.: *Enantioselective pharmacodynamics of the nonsteroidal antiinflammatory drug ketoprofen: in vitro inhibition of human platelet cyclooxygenase activity.* Chirality. 4:484-487, 1992b.

Hayball, P.J., Nation, R.L., Bochner, F., Newton, J.L., Massey-Westropp, R.A. and Hamon, D.P.G.: *Plasma protein binding of ketoprofen enantiomers in man: Method development and its application.* Chirality. 3:460-466, 1991.

Hayball, P.J., Nation, R.L., Bochner, F. and Le Leu, R.K.: *Enantiospecific analysis of ketoprofen in plasma by high-performance liquid chromatography.* J. Chromatogr. 570:446-452, 1991.

Heyada, M.A. and Sawchuk, R.J.: *Modeling the enhanced uptake of zidovudine (AZT) into cerebrospinal fluid. 1. Effect of probenecid.* Pharm. Res. 7:332-338, 1990.

Heyada, M.A. and Sawchuk, R.J.: *Effect of probenecid on the renal and nonrenal clearances of zidovudine and its distribution into cerebrospinal fluid in the rabbit.* J. Pharm. Sci. 78:716-722, 1989.

Hoffman, A. and Levy, G.: *Gender differences in the pharmacodynamics of barbiturates in rats.* Pharm. Res. 6:976-981, 1989.

Hooper, W.D. and Qing, M.S.: *The influence of age and gender on the stereoselective metabolism and pharmacokinetics of mephobarbital in humans.* Clin. Pharmacol. Ther. 48:633-640, 1990.

Horton, T.L. and Pollack, G.M.: *Enterohepatic recirculation and renal metabolism of morphine in the rat.* J. Pharm. Sci. 80:1147-1152, 1991.

Houghton, G.W., Dennis, M.J., Rigler, E.D. and Parsons, R.L.: *Urinary pharmacokinetics of orally administered ketoprofen in man.* Eur. J. Drug Metabol. Pharmacokinet. 9:201-204, 1984.

Hutt, A.J. and Caldwell, J.: *The importance of stereochemistry in the clinical pharmacokinetics of the 2-arylpropionic acid nonsteroidal antiinflammatory drugs.* Clin. Pharmacokinet., 9:371-373, 1984.

Hutt, A.J. and Caldwell, J: *The metabolic chiral inversion of 2-arylpropionic acids - a novel route with pharmacological consequences.* J. Pharm. Pharmacol. 35:693-704, 1983.

Ishizaki, T., Sasaki, T., Suganuma, T., Horai, Y. and Chiba, K.: *Pharmacokinetics of ketoprofen following single oral, intramuscular and rectal doses and after repeated administration.* Eur. J. Clin. Pharmacol. 18:407-414, 1980.

Iwakawa, S., He, X., Hashimoto, S., Volland, C., Benet, L.Z. and Lin, E.T.: *Stereoselective disposition of ketoprofen in rats.* Drug Metabol. Disp. 19:717-718, 1991.

Jack, D.S., Rumble, R.H., Davies, N.W. and Francis, H.W.: *Enantiospecific gas chromatographic-mass spectrometric procedure for the determination of ketoprofen and ibuprofen in synovial fluid and plasma: application to protein binding studies.* J. Chromatogr. 584:189-199, 1992.

Jamali, F., Russell, A.S., Foster, R.T. and Lemko, C.: *Ketoprofen pharmacokinetics in humans: evidence of enantiomeric inversion and lack of inversion.* J. Pharm. Sci. 79:460-461, 1990.

Jamali, F. and Brocks, D.: *Clinical pharmacokinetics of ketoprofen and its enantiomers.* Clin. Pharmacokinet. 19:197-217, 1990.

Jamali, F., Mehvar, R. and Pasutto, F.: *Enantioselective aspects of drug action and disposition: Therapeutic pitfalls.* J. Pharm. Sci. 78:695-715, 1989.

Jamali, F.: *Pharmacokinetics of enantiomers of chiral nonsteroidal antiiflammatory drugs.* Eur. J. Drug Metabol. Pharmacokinet. 13:1-9, 1988.

Jaussaud, P., Bellon, V., Besse, S., Courtot, D. and Delatour, P.: *Enantioselective pharmacokinetics of ketoprofen in horses.* J. Vet. Pharmacol. Ther. 16:373-376, 1993.

Jefferies, T.M., Thomas, W.O.A. and Parfitt, R.T.: *Determination of ketoprofen in plasma and urine by high performance liquid chromatography.* J. Chromatogr. 162:122-124, 1979.

Julou, L., Guyonnet, J.C., Ducrot, R., Fournel, J. and Pasquet, J.: *Ketoprofen (19583 R.P.): Main pharmacological properties.* Scand J. Rheumatol. Suppl. 14:33-44, 1976.

Kanai, S., Kitani, K., Sato, Y. and Nokubo, M.: *The age-dependent decline in the biliary transport maximum of conjugated sulfobromophthalein in the rat.* Arch. Gerontol. Geriatr. 7:1-8, 1988.

Kane, R.E. and Chen, L.J.: *Influence of gonadal hormones upon rat hepatic acetaminophen sulfotransferases.* Drug Metabol. Disp. 15:725-728, 1987.

Kepler, K.L., Standifer, K.M., Paul, D., Kest, B., Pasternak, G.W. and Bodnar, R.J.: *Gender effects and central opiod analgesia.* Pain. 45:87-94, 1991.

Knudsen-Randolph, L. and Ciminera, J.L.: *Statistics.* In Remington's Pharmaceutical Sciences ed. by A. Osol, G.D. Chase *et al.*, pp. 119-122, Mack Printing Company, PA, 1980.

Lin, J.H., Cocchetto, D.M. and Duggan, D.E.: *Protein binding as a primary determinant of the clinical pharmacokinetic properties of nonsteroidal antiinflammatory drugs.* Clin. Pharmacokinet. 12:402-432, 1987.

Liversidge, G.: *Ketoprofen.* Anal. Profiles Drug Subst. 10:443-471, 1981.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: *Protein measurement with the folin phenol reagent.* J. Biol. Chem. 193:265-275, 1951.

Macdonald, J.I., Herman, R.J. and Verbeeck, R.K.: *Sex-difference and the effects of smoking and oral contraceptive steroids on the kinetics of diflunisal.* Eur. J. Clin. Pharmacol. 38:175-179, 1990.

Mayer, J.M.: *Stereoselective metabolism of antiinflammatory 2-arylpropionates.* Acta. Pharm. Nord. 2:197-216, 1990.

Meerman, J.H.N., Nijland, C. and Mulder, G.: *Sex differences in sulfation and glucuronidation of phenol, 4-nitrophenol and N-hydroxy-2-acetylaminofluorene in the rat in vivo.* Biochem. Pharmacol. 36:2605-2608, 1987.

Meffin, P.J., Sallustio, B.C., Purdie, Y.J. and Jones, M.E.: *Enantioselective disposition of 2-arylpropionic acid nonsteroidal antiinflammatory drugs. 1. 2-phenylpropionic acid disposition.* J. Pharmacol. Exp. Ther. 238:280-287, 1986.

Meffin, P.J., Zilm, D.M. and Veenendaal, J.R.: *A renal mechanism for the clofibric acid-probenecid interaction.* J. Pharmacol. Exp. Ther. 227:732-734, 1983.

Mehlisch, D.R.: *Double-blind crossover comparison of ketoprofen, naproxen and placebo in patients with primary dysmenorrhea.* Clin. Ther. 12:398-409, 1990.

Melethil, S. and Conway, W.D.: *Urinary excretion of probenecid and its metabolites in humans as a function of dose.* J. Pharm. Sci. 65:861-865, 1976.

Menzel, S., Beck, W.S., Brune, K. and Geisslinger, G.: *Stereoselective biliary excretion of 2-arylpropionates in rats.* Chirality. 5:422-427, 1993.

Menzel-Soglowek, S., Geisslinger, G. and Brune, K.: *Stereoselective high performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral α_1-acid glycoprotein column.* J. Chromatogr. 532:295-303, 1990.

Merck Index. *Ketoprofen.* ed. by M. Windholz, p. 762, Merck & Co., Inc., 1983.

Miners, J.O., Robson, R.A. and Birkett, D.J.: *Gender and oral contraceptive steroids as determinants of drug glucuronidation: Effects on clofibric acid elimination.* Br. J. Clin. Pharmacol. 18:240-243, 1984.

Morikawa, M., Kojima, T., Inoue, M. and Tsuboi, M.: *Sex difference in the effect of aspirin on rat platelet aggregation and arachidonic acid metabolism.* Japan J. Pharmacol. 37:317-323, 1985.

Moseley, R.H.: *Bile Secretion.* In Textbook of Gastroenterology Vol 1 ed. by T. Yamada, D.H. Alpers, C. Owyang, D.W. Powell, F.E. Silverstein, pp. 314-333, J.B. Lippincott Company, PA, 1991.

Mulder, G.J.: *Sex differences in drug conjugation and their consequences for drug toxicity. Sulfation, glucuronidation and glutathione conjugation.* Chem. Biol. Interactions. 57:1-15, 1986.

Muller-Oerlinghausen, B. and Kunzel, B.: *Sex differences in the activity of rat liver UDPG-dehydrogenase.* Life Sci. 7:1129-1133, 1968.

Munger, K.A. and Blantz, R.C.: *Cyclooxygenase-dependent mediators of renal hemodynamic function in female rats.* Am. J. Physiol. 258:F1211-F1217, 1990.

Munger, K.A. and Baylis, C.: *Sex differences in renal hemodynamics in rats.* Am. J. Physiol. 254:F223-F231, 1988.

Nielsen, B., Sorenson, A.W., Szabo, L., Pedersen, E. and Scharff, A.: *Kanamycin serum half-life and renal function: age and sex.* Danish Med. Bull. 20:144-149, 1973.

Oda, Y., Asakawa, N., Yoshida, Y. and Sato, T.: *On-line determination and resolution of the enantiomers of ketoprofen in plasma using coupled achiral-chiral high performance liquid chromatography.* J. Pharm. Biomed. Anal. 10:81-87, 1992.

Oda, Y., Asakawa, N., Abe, S., Yoshida, Y. and Sato, T.: *Avidin protein-conjugated column for direct injection analysis of drug enantiomers in plasma by high performance liquid chromatography.* J. Chromatogr. 572:133-141, 1991.

Ollagnier, M., Decousus, H., Cherrah, Y., Levi, F., Mechkouri, M., Queneau, P. and Reinberg, A.: *Circadian changes in the pharmacokinetics of oral ketoprofen.* Clin. Pharmacokinet. 12:367-378, 1987.

Perrin, A., Milano, G., Thyss, A., Cambon, P. and Schneider, M.: *Biochemical and pharmacological consequences of the interaction between methotrexate and ketoprofen in the rabbit.* Br. J. Cancer. 62:736-741, 1990.

Populaire, P., Terlain, B., Pascal, S., Decouvelaere, B. and Renard, A.: *Comportement biologique: taux seriques excretion et biotransformation de l'acide (benzoyl-3-phenyl)-2 propionique ou ketoprofene chez l'animal et chez l'homme.* Ann. Pharm. Franc. 31:735-749, 1987.

Rendic, S., Sunjic, V., Kajfez, F., Blazevic, N. and Alebic-Kolbah, T.: *Resolution of (±)-α-(3-benzoylphenyl-)-propionic acid (Ketoprofen) and diastereomeric interaction of its enantiomers with some biological systems.* Chimia. 29:170-172, 1975.

Rendic, S., Alebic-Kolbah, T., Kajfez, F. and Sunjic, V.: *Stereoselective binding of (+)- and (-)-α-(benzoylphenyl) propionic acid (ketoprofen) to human serum albumin.* Il Farmaco. Ed.Sci. 35:51-59, 1980.

Rollins, D.E. and Klaassen, C.D.: *Biliary excretion of drugs in man.* Clin. Pharmacokinet. 4:368-379, 1979.

Roskos, L.K. and Boudinot, F.D.: *Effects of dose and sex on the pharmacokinetics of piroxicam in the rat.* Biopharm. Drug Disp. 11:215-225, 1990.

Runkel, R., Mroszczak, E., Chaplin, M., Sevelius, H. and Segre, E.: *Naproxen-probenecid interaction*. Clin. Pharmacol. Ther. 24:706-713, 1978.

Rush, G.F., Newton, J.F. and Hook, J.B.: *Sex differences in the excretion of glucuronide conjugates: The role of intrarenal glucuronidation*. J. Pharmacol. Exp. Ther. 227:658-662, 1983.

Sallustio, B.C., Purdie, Y.J., Whitehead, A.G., Ahern, M.J. and Meffin, P.J.: *The disposition of ketoprofen enantiomers in man*. Br. J. Clin. Pharmacol. 26:765-770, 1988.

Sallustio, B.C., Abas, A., Hayball, P.J., Purdie, Y.J. and Meffin, P.J.: *Enantiospecific high-performance liquid chromatographic analysis of 2-phenylpropionic acid, ketoprofen and fenoprofen*. J. Chromatogr. 374:329-337, 1986.

Satterwhite, J.H. and Boudinot, F.D.: *Pharmacokinetics of ketoprofen in rats: Effect of age and dose*. Biopharm. Drug Disp. 13:197-212, 1992.

Schenkman, J.B., Frey, I., Remmer, H. and Estabrook, R.W.: *Sex differences in drug metabolism by rat liver microsomes.* Mol. Pharmacol. 3:516-525, 1967

Seitz, H.K., Xu, Y., Simanowski, U.A. and Osswald, B.: *Effect of age and gender on in vivo ethanol elimination, hepatic alcohol dehydrogenase activity and NAD+ availability in F344 rats.* Res. Exp. Med. (Berlin). 192:205-212, 1992.

Selen, A., Amidon, G.L. and Welling, P.G.: *Pharmacokinetics of probenecid following oral doses to human volunteers.* J. Pharm. Sci. 71:1238-1242, 1982.

Singh, N.N., Pasutto, F.M., Coutts, R.T. and Jamali, F.: *Gas chromatographic separation of optically active antiinflammatory 2-arylpropionic acids using (+)- or (-)-amphetamine as a derivatizing agent.* J. Chromatogr. 378:125-135, 1986.

Sjoberg, P., Ekman, L. and Lundqvist, T.: *Dose and sex-dependent disposition of ketoconazole in rats.* Arch. Toxicol. 62:177-180, 1988.

Skett, P.: *Biochemical basis of sex differences in drug metabolism.* Pharmacol. Ther. 38: 269-304, 1988.

Skett, P.: *Hormonal regulation and sex differences of xenobiotic metabolism.* Prog. Drug Metabol. 10:85-139, 1987.

Skett, P., Mode, A., Rafter, J., Sahlin, L. and Gustafsson, J.A.: *The effects of gonadectomy and hypophysectomy on the metabolism of imipramine and lidocaine by the liver of male and female rats.* Biochem. Pharmacol. 29:2759-2762, 1980.

Shibukawa, A., Terakita, A., He, J.Y. and Nakagawa, T. *High-performance frontal analysis - high-performance liquid chromatographic system for stereoselective determination of unbound ketoprofen enantiomers in plasma after direct sample injection.* J. Pharm. Sci. 81:710-715, 1992.

Singer, L., Imbs, J.L. and Danion, J.M.: *Risk of lithium poisoning during combination with nonsteroidal antiinflammatory drugs.* Therapie. 36:323-326, 1981.

Smith, P.C., Lagendijk, P.N.J., Bosso, J.A. and Benet, L.Z.: *Effect of probenecid on the formation and elimination of acyl glucuronides: Studies with zomepirac.* Clin. Pharmacol. Ther. 38:121-127, 1985.

Sorgel, F., Behyl, F.E. and Mutschler, E.: *Inhibition of uridine diphosphate glucuronyltransferase caused by furosemide.* Experientia. 36:861-863, 1980.

Sorrentino, D., Licko, V. and Weisiger, R.A.: *Sex differences in sulfobromophthalein-glutathione transport by perfused rat liver.* Biochem. Pharmacol. 37:3119-3126, 1988.

Spahn, H. and Benet, L.Z. *Influence of probenecid on the glucuronidation of naproxen enantiomers by hepatic UDP-glucuronyltransferase in rat liver microsomes.* Pharm. Res. Suppl. 4:S111, 1987.

Spahn, H., Spahn, I. and Benet, L.Z.: *Probenecid-induced changes in the clearance of carprofen enantiomers: A preliminary study.* Clin. Pharmacol. Ther. 45:500-505, 1989.

Spahn, H., Iwakawa, S., Benet, L.Z. and Lin, E.T.: *Influence of probenecid on the urinary excretion rates of the diastereomeric benoxaprofen glucuronides.* Eur. J. Drug Metabol. Pharmacokinet. 12:233-237, 1987.

Spiers, C.J.: *Comparison of the safety of several nonsteroidal antiinflammatory drugs currently or formerly marketed in the United Kingdom.* J. Clin. Pharmacol. 28:S8-S12, 1988.

Steele, R.G.O. and Torrie, J.H.: *Principles and Procedures of Statistics.* McGraw-Hill, NY, pp 453, 1960.

Strafanger, G., Larsen, H.W., Hansen, H. and Sorenson, K.: *Pharmacokinetics of ketoprofen in patients with chronic renal failure.* Scand. J. Rheumatol. 10:189-192, 1981.

Sunshine, A., Olson, N.Z., Zighelboim, I. and De Castro, A.: *Ketoprofen, acetaminophen plus oxycodone, and acetaminophen in the relief of postoperative pain.* Clin. Pharmacol. Ther. 54:546-555, 1993.

Sunshine, A. and Olson, N.Z.: *Analgesic efficacy of ketoprofen in postpartum, general surgery, and chronic cancer pain.* J. Clin. Pharmacol. 28:S47-S54, 1988.

Tanaka, Y., Kadoh, Y. and Ishikawa, H.: *Effects of hypophysectomy and testosterone treatment on the urinary excretion of zenarestat in rats.* Xenobiotica. 22:51-55, 1992.

Tanaka, Y., Deguchi, Y., Ishi, I and Terai, T.: *Sex differences in excretion of zenarestat in rat.* Xenobiotica. 21:1119-1125, 1991.

Thyss, A., Milano, G., Kubar, J., Namer, M. and Schneider, M.: *Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen.* Lancet. 8475:256-258, 1986.

Trnavska, Z. and Trnavsky, K.: *Sex differences in the pharmacokinetics of salicylates.* Eur. J. Clin. Pharmacol. 25:679-682, 1983.

Tucker, G.T and Lennard, M.S: *Enantiomer specific pharmacokinetics.* Pharmacol. Ther. 45:309-329, 1990.

Upton, R.A., Williams, R.L., Buskin, J.N. and Jones, R.M.: *Effects of probenecid on ketoprofen kinetics.* Clin. Pharmacol. Ther. 31:706-712, 1982.

Upton, R.A., Buskin, J.N., Guentert, T.W., Williams, R.L. and Riegelman, S.: *Convenient and sensitive high performance liquid chromatography assay for ketoprofen, naproxen and other allied drugs in plasma or urine.* J. Chromatogr. 190:119-128, 1980.

Veys, E.: *20 years experience with ketoprofen.* Scand. J. Rheumatol. Suppl. 90:3-44, 1991.

Vonk, R.J., Van Doorn, A.B.D. and Strubbe, J.H.: *Bile secretion and bile composition in the freely moving unanesthetized rat with a permanent biliary drainage: influence of food intake on bile flow.* Clin. Sci. Mol. Med. 55:253-259, 1978.

Wallis, W.J. and Simkin, P.A.: *Antirheumatic drug concentrations in human synovial fluid and synovial tissue.* Clin. Pharmacokinet. 8:496-522, 1983.

Waxman D.J. : *Interactions of hepatic cytochromes P-450 with steroid hormones.* Biochem. Pharmacol. 37:71-84, 1988.

Waynforth, H.B.: *Experimental and Surgical Technique in the Rat.* Academic Press, London, 1980.

Wilbrink, B., Van der Veen, M.J., Huber, J, Van Roy, J.L.A.M. and Huber-Bruning, O.: *In vitro influence of ketoprofen on the proteoglycan metabolism of human normal and osteoarthritis cartilage.* Agents Actions. 32:154-159, 1991.

Williams, R.L. and Upton, R.A.: *The clinical pharmacology of ketoprofen.* J. Clin. Pharmacol. 28:S13-S22, 1988.

Williams, R.L., Upton, R.A., Buskin, J.N. and Jones, R.M.: *Ketoprofen-aspirin interactions.* Clin. Pharmacol. Ther. 30:226-231, 1981..

Wilson, K.: *Sex related differences in drug disposition in man.* Clin. Pharmacokinet. 9:189-202, 1984.

Wilson, K., Oram, M., Horth, C.E. and Burnett, D.: *The influence of menstrual cycle on the metabolism and clearance of methaqualone.* Br. J. Clin. Pharmacol. 14:333-339, 1982.

Witkamp, R.F., Lohuis, J.A.C.M., Nijmeijer, S.M., Kolker, H.J., Noordhoek, J. and Van Miert, A.S.J.P.A.M.: *Species and sex-related differences in the plasma clearance and metabolite formation of antipyrine. A comparative study in four animal species: cattle, goat, rat and rabbit.* Xenobiotica. 21:1483-1492, 1991.

Wright, M.R., Davies, N.M., Russell, A.S. and Jamali, F.: *Influence of enantiomers of nonsteroidal antiinflammatory drugs (NSAIDs) on intestinal permeability as measured with ^{51}Cr-EDTA.* Pharm. Res. Suppl. 10:S307, 1993.

Yagi, M., Shibukawa, A. and Nakagawa, T.: *Direct injection analysis of ketoprofen enantiomers in plasma using a column switching high performance liquid chromatography system.* Chem. Pharm. Bull. (Tokyo). 38:2513-2517, 1990.

Zeidler, H.: *Epidemiology of NSAID induced gastropathy.* J. Rheumatol. 18, S28:2-5, 1991.

Zeng, Q.Q., Jee, W.S.S., Ke, H.Z. and Wechter, W.J.: *S-ketoprofen inhibits tenotomy-induced bone loss and dynamics in weanling rats.* Bone Miner. 21:203-218, 1993.